University of Alberta

Callosal Morphology Differences in Major Depressive Disorder Associated with Adverse Childhood Experiences

by

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Dedication

I would like to thank all of the persons who provided encouragement and support throughout this project. In particular, appreciation goes to my supervisors, Drs. Nick Coupland and Kathy Hegadoren. Thank you for guidance with the project, research and introducing me to the world of academia. I'm grateful for the chance that I was given to learn about and participate in such a variety of interesting, multi-disciplinary projects. Dr. Coupland, thank you for allowing me to make many decisions but providing many helpful suggestions and pieces of advice along the way. Dr. Hegadoren, thank you for opening my eyes to the multi-faceted life of an academic and leading the way with integrity. Without a doubt, my experience during this degree will play an important role in what I choose to pursue in the coming years.

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Abstract

The advent of magnetic resonance imaging (MRI) technology has made it possible to non-invasively visualize structures in the human brain. Due to its integral role in brain function the corpus callosum is a prime candidate for studies investigating neuropsychiatric pathology.

This thesis presents a study of the corpus callosum in patients diagnosed with major depressive disorder (MDD). While research to date has only yielded ambiguous results, studies in pediatric populations have been linked with childhood maltreatment, a risk factor for MDD. This study uses MRI to examine callosal morphology in adult MDD and control groups. The patient group was further divided into subgroups by history of childhood maltreatment.

This data suggests that 1) there are differences in the morphology of callosal subdivisions between patient and control groups and that 2) these differences are possibly related to adverse childhood experiences of the patient group.

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List of Abbreviations

AAMs	Active Appearance Models
AC-PC	A Line between the Anterior and Posterior Commissures, Used to Orient Brain Images
ACC-PCC	A Line between the Anterior- and Posterior-most Points of the Corpus Callosum
ADHD	Attention Deficit Hyperactivity Disorder
AgCC	Agenesis of the Corpus Callosum
BDNF	Brain Derived Neurotrophic Factor
BPD	Borderline Personality Disorder
CC	Corpus Callosum
CCA	Corpus Callosum Area
CIT	Childhood Interpersonal Trauma
CPA	Childhood Physical Abuse
CSA	Childhood Sexual Abuse
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CTQ	Childhood Trauma Questionnaire
DTI	Diffusion Tensor Imaging
EAN	(Childhood) Emotional Abuse/Neglect
EEG	Electroencephalogram
FA	Fractional Anisotropy
FOV	Field of View
GM	Gray Matter

HPA Axis	Hypothalamic-Pituitary-Adrenal Axis
ICV	Intracranial Volume
LH	Left Handed
MDD	Major Depressive Disorder
MR, MRI	Magnetic Resonance, Magnetic Resonance Imaging
MSS	Mid Sagittal Slice
NGF	Nerve Growth Factor
NMR	Nuclear Magnetic Resonance
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PTSD	Posttraumatic Stress Disorder
RF	Radio Frequency
RH	Right Handed
ROI	Region of Interest
SNR	Signal to Noise Ratio
SPA	(Childhood) Sexual/Physical Abuse
WM	White Matter
vmPFC	Ventromedial Prefrontal Cortex

*Note: The author recognizes the differences between the terms sex and gender and every effort was made to accurately use these terms. Sex refers to biological characteristics such as anatomical and physiological differences while gender refers to the array of socially constructed roles and relationships, personality traits, behaviours, values and influence that society ascribes to two sexes based on a differential basis. Further information on these terms can be found on the website of the Canadian Institutes of Health Research (http://www.cihr-irsc.gc.ca/e/32019.html).

Chapter 1

Introduction

Depression is a serious disorder that occurs in people of all ages, genders and backgrounds, and is projected to become the second leading cause of disability worldwide the year 2020 [1]. The precise cause of depression is unknown, but researchers have been able to identify certain factors which seem to increase the risk of developing this disease (see Appendix A2 for a list of risk factors). One of these factors is adverse events in childhood and recent studies have explored the idea that early adversity can affect subsequent neurological development. These early events include sexual, physical and emotional abuse, as well as neglect.

Many efforts have been made to determine the etiology of depression, and using neuroimaging techniques, researchers have been able to identify differences in brain structure in major depressive disorder (MDD) patients compared to controls. Most of these differences have been found in gray matter (GM) regions such as the hippocampus, anterior cingulate and frontal cortex. By contrast, and perhaps partly due to difficulty in the ability to consistently parcellate (or neatly segment) it, research findings in white matter (WM) regions have been limited. In the nervous system, WM represents the material through which nerve impulses pass through on their paths between gray matter structures. Made up of myelinated fibres, WM makes up the bulk of the deep parts of the brain and is the basis for communication throughout the brain.

In the search for WM pathology in MDD, one natural structure to examine is the corpus callosum (CC), the largest of the commissural WM structures found in the brain.

The CC has been shown to be vulnerable to stress during development, suggesting that MDD risk factors such as childhood maltreatment could influence callosal structure, with subsequent impact on interhemispheric communication and integrative functions.

The purpose of the current research is to help identify relationships between callosal morphology, MDD and their connection to childhood adversity. To this end, the current investigation studies a comparative patient and control sample using magnetic resonance imaging (MRI) to examine the anatomy of the CC. The anatomical MRI results for the corpus callosum are also examined for correlations with prior diffusion tensor imaging (DTI) and GM volumetric data gathered in the same sample. Combining these techniques provides the opportunity to examine the results and determine relationships from multiple perspectives. Compared to using a single imaging approach, or single type of data, this may aid in more detailed interpretation of the results.

Due to methodological issues and difficulties in segmenting the CC, studies to date have shown mixed results when trying to confirm the presence (and establish the nature) of callosal changes in MDD. In support of the literature in which such changes have been detected, this investigation finds that there are differences in the callosal morphologies of patients related to histories of childhood maltreatment. While these findings stand to be corroborated by future research and interpretation is preliminary, the establishment of links between callosal morphology, childhood maltreatment and MDD indicates the presence of an important relationship between brain development, experiential factors and resulting pathophysiology.

This study presents a starting point for subsequent research and the techniques used within demonstrate promise in their ability to further knowledge in the field. The implications of this research are multidisciplinary as not only are the fields of psychiatry and

neuroimaging highlighted, but psychosocial considerations are also raised with the inclusion of the factor of childhood adverse events.

To facilitate discussion, the body of this thesis is divided into several sections. The second chapter is an overview of imaging techniques used in the measurement of the corpus callosum. This section briefly introduces MRI and DTI, tools which have made the current investigation possible. The third chapter focuses on the corpus callosum itself, identifying its properties and development, while providing a review of considerations that need to be taken into account when measuring the CC. Included in this chapter is a discussion of which specific techniques are advantageous in segmenting callosal regions. The following chapter examines depression and the specific research that has been conducted on the CC in MDD, and relates that to research on childhood maltreatment, establishing the basis of this thesis' focus. Chapter five is a short section which clearly outlines the research question, while the sixth chapter describes the study parameters— the methods used to address the question and the rationale for the design choices. In this section, the subject group, image acquisition and callosal segmentation protocol are described. The seventh chapter discusses the findings of the project and offers insight into the possible significance of the findings. The thesis is concluded by a final chapter in which contributions to the field, study limitations and future research avenues are detailed.

Chapter 2

What is Brain Imaging?

In vivo imaging techniques have evolved greatly in recent years, extending visualization of the human brain. Brain imaging refers to a group of technologies that allow the observation of the human brain in living human subjects [2]. Historically, brain measurements were performed in the context of post-mortem morphological or histological evaluations, requiring brain tissue to be directly handled and processed. However, non-invasive brain imaging has greatly increased the power of neuroscientific research. Imaging techniques have been especially useful in researching mental disorders, as they have allowed contrasts between brains of patient and control groups. These comparisons allow the detection of brain changes and abnormalities between the different groups and may indicate which areas of the brain are at the root of underlying disease pathology. What follows is a brief overview of several of the techniques used currently in the field, as well as in the current investigation. The two techniques of MRI and DTI will be the focus of the discussion.

2.0 A Brief Description of Magnetic Resonance Imaging

In use only since the early 1970s [3], MRI is a technology used to obtain structural images of the human body. Its utility has made it the primary modality for imaging the human brain. Due to the non-invasive, efficient nature of the procedure, it has largely replaced X-rays, computed tomography (CT) scans and positron emission tomography (PET) scans for many purposes. In addition to eliminating the need for ionizing radiation

and radionuclide tracers, an advantage that MRI has over traditional imaging techniques is that it can obtain images in all planes (sagittal, coronal, and transverse), providing a three dimensional image of the brain. In addition, MRI techniques produce images with superb gray-white resolution, allowing detailed morphometric studies of brain structure, an important factor when it comes to detecting small changes in the brain [2]. The ability to achieve these high-level contrasts makes MRI a powerful tool well suited for neurological research.

How Does it Work?

As the name implies, this technique uses properties of magnetic resonance to obtain images of the brain. In brief, H^1 atoms in the brain are aligned by placing them in a strong magnetic field. The atoms are then excited by the application of a second oscillating magnetic field, called a radio frequency (RF) pulse. Following excitation, magnetization returns to its equilibrium state through an exponential decay process referred to as relaxation, releasing energy. This energy is measured as the MRI signal and subtle differences in this signal are detected and processed by a computer. The signal detected depends on a combination of relaxation times and properties of the tissue to be imaged [4].

The manipulation of the relaxation times (by changing the frequencies and pulse patterns of the secondary excitatory field) permit researchers to emphasize different structures during visualization. The brain is commonly visualized with either T1 (the recovery of longitudinal magnetization) or T2 (the loss of transverse magnetization) weightings [4]. Subsequently, these manipulations produce images which allow researchers to detect structural changes or abnormalities in particular areas of interest. The current study is interested mainly in WM and the CC is prominently visualized in T1- weighted scans

(see Figure 2.0). Table 2.0 indicates how different brain tissues appear when visualized when T1 or T2 weighted.

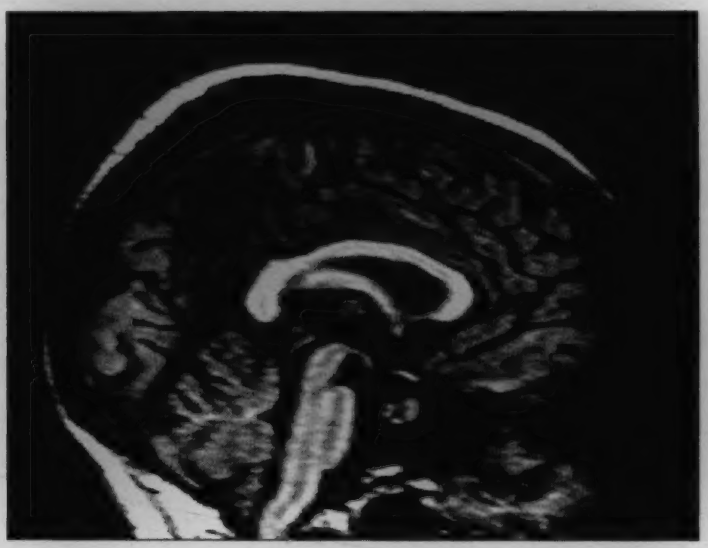

Figure 2.0: T1 Weighted Sagittal Magnetic Resonance Image

	T1 Weighting	*T2 Weighting*
White Matter	Light	Dark
Gray Matter	Dark	Light
Cerebrospinal Fluid	Very Dark	Very Bright
Bone	Very Dark	Very Dark

Table 2.0: The Visual Properties of T1 and T2 Weighted Images

One important characteristic of MRI techniques is that the final output of information is a condensed form of the actual brain shape [5]. This is a great benefit as it allows a relatively quick examination resulting in a small computer file which can be processed and analyzed as desired. At the same time, the condensed information is a limitation because a significant amount of biological information is lost [5]. Nevertheless,

MRI has been increasingly used in research on a variety of mental disorders and continues to hold promise in studying the etiology of neuropsychiatric disorders. Techniques building on basic MRI technology, such as functional magnetic resonance imaging (fMRI) and DTI are increasing researchers' abilities to visualize the brain and its irregularities in different pathologies.

2.1 A Brief Description of Diffusion Tensor Imaging

Introduced in the mid- 1990s, DTI is a relatively new imaging technique that provides detailed information that cannot be obtained from conventional MRI approaches. DTI is actually a variation of MRI technique that allows visualization of the directionality of water diffusion, particularly along WM tracts [6]. If there are no barriers to the movement of water molecules, this movement is said to be random or isotropic. White matter (WM) consists of tracts made up of densely packed axons, as well as various types of neuroglial cells [7]. Due to the properties of physical diffusion, it is likely that a water molecule will move principally along the axis of a fiber, with a low probability that a molecule would cross the lipid-containing myelin sheath or axonal membrane.

Using these microstructural properties, DTI effectively provides information about two properties of water diffusion, measuring diffusion anisotropy and direction [7]. These factors are determined by detecting the translational motion of water molecules as they are affected by thermal energy [8]. Isotropic movement refers to the random movement of molecules in a medium (for example, a drop of ink spreading in water). Anisotropic movement, or anisotropy, is the amount of deviation from randomness (a drop of ink diffusing along the grain of a piece of wood). Fractional Anisotropy (FA) combines the

measure of anisotropy with the measure of the primary direction of diffusion, and is found to be greatest in highly organized, directed WM tracts (see Figure 2.1). As a

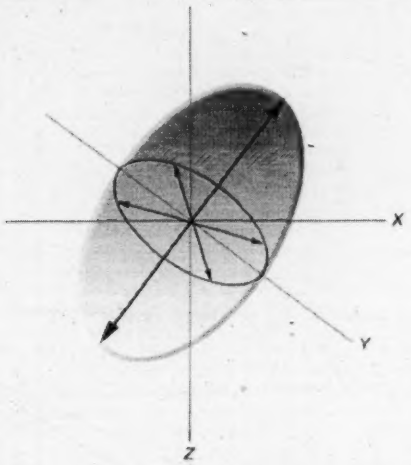

Figure 2.1: Diffusion Tensor Imaging: A Diffusion Ellipsoid

From diffusion measurements along multiple axes, the orientation of the longest axes and FA of a pixel can be found. The more polar the shape of the ellipsoid, the stronger the FA. The more spherical the ellipsoid, the more isotropic the diffusion (Adapted from Mori and Zhang [5]).

result, FA can be used as an estimate of axonal organization in the brain- the higher the FA, the stronger the directionality of the diffusion [5]. These principles are also used to identify the directionality of WM fibers and tract orientation or organization. Three dimensional images and maps of tracts can then be constructed (see Appendix D, Figure D.0).

There are several factors that can lead to changes in anisotropy, such as axonal density, caliber and myelination. Studies have shown that FA increases rapidly in early years, consistent with the idea of increasing WM myelination [9]. It has been shown that FA of WM reaches an asymptote in early adulthood (20s and 30s) and remains constant until late life (70s and 80s), when there seems to be a breakdown of tissue [9, 10, 11].

Although at this time it is not possible to definitively say if and how DTI results apply directly to brain function [12], it has been shown in preliminary studies that FA

microstructure is predictive of function [13, 14]. Studies in adolescent and adult populations have related FA to performance on cognitive tasks, with higher FA values leading to better performance [15, 16]. Even though DTI is still developing as a tool and its interpretation is not definitive, advances in the technology may be able to increase understanding of neural connectivity and relate cognitive function to cortical connections [5]. In particular, the combination of DTI with functional MRI may lead to a more refined understanding of CC function [13, 17]. There are high expectations for DTI, as it is currently the only available method that is able to non-invasively study WM architecture [5].

Chapter 3

The Corpus Callosum

The corpus callosum has been the subject of extensive research, but due to conflicting results throughout the literature, many of its properties remain unverified. It is the main interhemispheric fibre tract in the brains of placental mammals [18, 19]. Historically, limited models of brain function have translated into limited understanding of callosal properties. It was originally thought that the CC served mainly as a mechanical support to the brain, but results of callosotomy (surgical procedure that disconnects the cerebral hemispheres) studies led researchers to begin ascribing communications to the callosal structure [20]. Initial anatomical observations such as the CC's central location, widespread connections, and density of connective nerve fibres (~200 million) suggested that it was the callosal structure that somehow 'unified' the brain as a whole [21]. The interconnected nature of callosal fibers, especially with the cerebral association cortices, suggested that the CC was also somehow involved in processes of higher function [21].

The study of epilepsy and its potential treatments was instrumental in the understanding of callosal function. Attempts to determine the cause of seizures led researchers to begin studying the CC and identifying it as commissural tissue. In the late 19th century, investigators found that the CC played a role in seizure spread in experimental animals, observing that stimulations to one side of the brain had contralateral limb effects [20]. Subsequent seizure studies in human subjects have allowed interpretations of communication deficits caused by callosal lesions, although it is possible that some portion of these deficits may be accounted for by the primary epileptic lesion [20, 22]. 'Split-brain'

studies continue to be explored in detail in the field of psychiatric research [23, 24]. In addition to the studies based on the idea of brain disconnection, other types of callosal pathology have helped identify callosal properties. Agenesis of the CC (AgCC) has been shown to result from several congenital syndromes and malformations are observed in combination with neural deficits [25]. These findings suggest that genes involved in CC development are also related to genes involved in the development of other brain areas [26]. Comparative studies of subjects with AgCC and controls using MRI, electroencephalogram and neuropsychological testing approaches continue to provide insight into healthy callosal function [25]. While undoubtedly important, the findings in epilepsy research and AgCC studies are tempered by the fact that there may be accompanying lesions or maldevelopment in other brain areas.

The results of the studies mentioned above, combined with research in normative populations, have outlined major roles for the CC in integrating motor, sensory and cognitive brain functions [24, 26]. The current chapter explores the properties of the CC as they are reflected in 'normal' subjects. The exploration reveals that 'normalcy' is not so easily classified and that the literature on the CC has been riddled with inconsistencies.

3.0 Callosal Properties and Function

An evolutionary standpoint may be useful in the discussion of the development of the CC as a brain structure. In acallosal mammals, communicative functions across the hemispheres are thought to be carried out largely by the anterior and hippocampal commissures. For smaller mammals with smaller brains, survival functions could be sufficiently carried out in this fashion [18]. Comparative research emphasizes that brain size has played a role in the development of the large commissural tract found in more 'highly

developed mammals' [18]. Research focusing on the size of the brain suggests that the presence of the CC may be related to the fact that sensory areas are larger, increasing the need for integrative connections [18, 26]. For example, the development of the isocortex and topographic sensory and motor surfaces has likely caused the proliferation of interhemispheric fibres, suggesting that timing constraints for interhemispheric transmission may be important in primary and secondary sensory areas. Not unlikely is the idea that with the development of a neocortex with areas of higher function, increased commissural tissue became necessary [18].

The discussion of evolutionary pressures leads to an exploration of development, the understanding of which is integral when it comes to studying or interpreting callosal changes or abnormalities. All reports of the development of the CC point to a complex process with a specific, fairly consistent timeline.

3.0.1 Development of the CC

Like the development of any brain region, the development of the CC can be affected by many factors, making it vulnerable to the environment or insult by other means [27]. The CC has been shown to be sensitive to substances such as alcohol and solvents and is also susceptible to WM diseases such as multiple sclerosis [27]. The majority of callosal development has been shown to occur at an early age. Development begins during the 12-16 week of the gestational period, and continues after birth. The nerve fibre connections passing through the CC are thought to be mainly completed before birth. Subsequent major changes of the CC take place during the 2nd and 3rd months of life, with an 'adult' appearance being achieved by the 8th month of life [27]. An increase in the number of axons and proportion of large myelinated axons increases the size of the CC. This development is

thought to be especially important in cognition, taking place anywhere from age four to age thirty [27, 28, 29]. Within that range, from age four to eighteen, it has been shown that while brain size remains fairly constant, CC boundaries become more regular and less complex [29]. There is some difficulty in characterizing CC development due to size variability between subjects, but it is consistently found that the most important period of CC development is at a young age [30]. Once mature (past the third and fourth decades), CC areas seem to remain stable, with little age related shrinkage through the seventh decade of life. Several studies using anatomical MRI have identified no significant correlation between CCA and adult age [31, 32, 33]. However, there are also studies which have found aging effects in those exceeding 55 years of age [34]. As previously stated, microstructural differences which cannot be detected on MR images (which measure size) may be present [9, 10, 11]. In either case, it is important to be able to relate the developmental course of the CC to changes in its morphology and subsequently to the events or factors which may have caused them.

3.0.2 Fibre Composition and Organization of the CC

It is thought that as the CC matures, communicative ability between the hemispheres becomes consolidated. Exactly how this information is transferred is a subject of ongoing study. It has been noted that even the slightest remaining connection may result in the preservation of substantial interhemispheric communications [20, 23]. Understanding basic anatomical organization of the CC informs understanding of studies measuring callosal characteristics. Intuitively, the first measure to examine is the holistic appearance of the CC, and this can be done using MRI technology. The apparent size and shape of the CC

depends largely on the fibers which make it up and particularly their number, size, packing density and degree of myelination [29].

At first glance, when visualized with most techniques, the CC appears homogeneous throughout and does not have any natural dividing landmarks (see Figure 3.0). In an effort to study the CC, investigators have attempted to organize it into several regions. The most common terms used to refer to the CC are from anterior to posterior- the genu, the body and the splenium, with more specific schemes further dividing these areas (see Table 3.0). Unfortunately, these sub-regions have not been standardized (an issue that will be discussed in section 3.1), but they still provide a set of common terms that can be generally applied and understood. The basis for these divisions stem from the idea that callosal fibres project to various areas throughout the hemispheres and are topographically organized with respect to the cortical regions they connect [18, 35]. DTI has allowed researchers to identify these fibres in greater detail but due to the newness of these techniques they have not yet been fully applied to the field (see Appendix D, Figure D.0).

Region	Anatomical Label	Corresponding Cortical Region
1 (Anterior)	Rostrum	Caudal/orbital prefrontal, inferior premotor
2	Genu	Prefrontal
3	Rostral Body	Premotor, supplementary motor
4	Anterior Midbody	Motor
5	Posterior Midbody	Somaesthetic, posterior parietal
6	Isthmus	Superior temporal, posterior parietal
7 (Posterior)	Splenium	Occipital, inferior temporal

Table 3.0: The Sub-Regions of the Corpus Callosum (Witelson, [51])

Figure 3.0: Sagittal MRI Image- The Homogeneous Corpus Callosum

Closer examinations have shown that the CC is comprised of both myelinated and unmyelinated axons, with a greater proportion of myelinated fibres [36]. These two main types of fibres are organized into sections which make up the callosal regions. In areas connecting primary and secondary sensorimotor areas there is a concentration of thick, highly myelinated, fast-conducting fibres [36]. Following cortical organization, the anterior midbody transfers primarily motor information, the posterior midbody transfers somatosensory information and the isthmus transfers auditory information [23]. Meanwhile, callosal regions connecting prefrontal and temperoparietal association areas carry a larger proportion of poorly myelinated, slow conducting fibers. With regards to location, the densities of thin fibres are seemingly highest in the anterior corpus callosum and isthmus/ anterior splenium. The large-diameter fibres are organized in a complementary pattern, found in the midbody and posterior splenium [36].

These findings seem to indicate that in primary and secondary sensory areas, there is a strong need for quick interhemispheric communications. In contrast, in the areas of 'higher function' the delay of communication can be longer [18]. This property appears relevant to the current discussion, as in many psychiatric disorders basic sensorimotor functions are not severely affected. However, specific functions depending on rapid interhemispheric transfer have not been a topic of extensive study so it cannot be conclusively stated that they are not also abnormal.

3.0.3 Anatomic and Functional Implications

The benefit of familiarity with callosal properties extends into surgical and anatomical applications. It has been long observed that people who have had partial or complete callosal sections present with a variety of different behavioural deficits, including anomia, hemi-alexia, aphasia, and apraxia [23]. These patient populations have allowed insight into the nature of interhemispheric communications made possible via the CC. Hemispheric specialization in visual perception, language, memory, and other areas of perception and cognition have been extensively studied [23].

Neurosurgical techniques can now make use of the knowledge of callosal development and projections in order to specify which areas to lesion [20, 37]. As an example, in epilepsy, sparing the splenium (and anterior commissure) has become common, as a section of the anterior two-thirds to three-quarters of the CC can alleviate drop attacks (spontaneous falls while walking or standing which can be caused by seizures) [20]. It has been observed that split-brain treatments can result in behavioural deficits, but cause and effect remains a gray area, due to the possibility of pre-existing cortical dysfunction. It may be that similar deficits found in other patient groups depend primarily on cortical dysfunction rather than the interhemispheric connections.

3.1 Issues in Callosal Measurement

The identifiable issues in callosal measurement stem from several fundamental complications which arise in the measurement of the CC. As with any brain structure, a reliable method of quantification is a necessary first step in order to compare and replicate results and to come to a reasonable consensus on the significance of findings. Currently, such agreements are difficult to find within the literature, as a variety of individual

differences within the normative population has led researchers to conflicting conclusions. Researchers face difficulty in determining callosal morphology due to issues such as sex, handedness and age differences between healthy individuals. Furthermore, unstandardized methodological strategies may also have contributed to inconsistent findings [33, 38, 39]. Therefore, subsequent research must make an effort to measure the CC with a consistent method.

3.1.0 The Midsagittal Slice

Figure 3.1: Gray's Figure #733: The Corpus Callosum From Above [145]

Measurement at the midsagittal slice (MSS) is necessary as all callosal fibres project throughout the cerebral hemispheres without established lateral boundaries (see Figure 3.1). Therefore, the most constant focus of callosal measurement is the MSS; parameters such as length, thickness and cross-sectional area (CCA) have been measured [40]. It is clear that the CC can be measured at the MSS, but additional difficulty is posed by the fact that although

different types of fibres run through the MSS, there are no visible anatomical landmarks that can be used to consistently divide the CC into different sub-regions. This has lead to the proposal of a number of different schemes to partition callosal sub-regions. Unfortunately, the proposed models of segmentation are not definitive and the 'best' way to segment the CC has yet to be determined [29, 41]. A further discussion of the MSS and of difficulties in measurement follows later in the current chapter (section 3.2)

3.1.1 Controversy and Gender Differences

Perhaps the most prominent point of contention in the callosal literature is sexual dimorphism. Even though focused research into the area of gender differences in the CC approaches its fourth decade, no consensus has been reached [39]. Results have been scattered in such a way that there are studies finding larger CCA in women than men, larger CCA in men than women, as well as no significant gender differences.

Data originally collected before 1910 from cadavers indicated that on average, males have larger brains and corpus callosa than females [39]. It has also been shown that even though the average brain size of men is larger than that of women by a single standard deviation, intelligence tests and metabolic rate per volume of tissue are found to be equal [39]. More recent research in the area of callosal gender differences was pioneered in a study suggesting the splenium of the CC as a site of significant gender difference [35]. This study found the splenium to be larger in women than men, and prompted many subsequent studies examining the CC in light of possible gender differences. Due to the prominence of the original study, the CC came to be on display to the public eye, and articles such as one in *Time* magazine generated explanations of perceived male-female difference in behaviour: "Often wider in the brains of women than in those of men, it may allow for greater cross-

talk between the hemispheres—possibly the basis for woman's intuition" [42]. Popular science aside, several reports arrived at similar conclusions, with women showing larger splenia than men [35, 43, 45], as well as equivalent or larger sized CCA after brain volume differences had been taken into account [44, 45]. Other studies have reported increased callosal areas in men [31], and others still report an absence of such differences [46, 47].

The reasons for these inconsistencies are varied, but may come down to issues in methodology. Some of these conflicting results may stem from the fact that total brain weight and brain/intracranial volume (ICV) were not taken into account in many studies. Even when considered, these measures cannot necessarily be directly compared as they may be affected by different developmental factors [32]. In addition, the lack of standardized definitions of the callosal subregions hampers gender comparative studies. It is also true that within the sexes, variation in brain and CC sizes exist [39]. For example, a larger man is likely to have a larger brain and corpus callosum than a smaller man. In an extensive meta-analysis examining 49 studies of sexual dimorphism in the CC, Bishop and Wahlsten [39] contend that aside from the allometrically logical finding that larger bodies would result in larger brains, and as a result larger CC, there is no significant physiological sex difference (see Appendix D, Table D.0). Moreover, the review states that there is no reason to believe that any such difference would necessarily be manifested as a behavioural or functional difference to the extent claimed or sensationalized by the media.

More recently, data from a large (n = 432) study have shown that female subjects have a smaller absolute total CCA than males, but a larger CCA ratio to ICV, and larger CCA-to-ICV ratios for each 1/3 along the length of the CC [48]. Other approaches to this topic of research are using other factors to search for relationships; for example, examining callosal gender differences as they are related specifically to psychiatric disorders [49]. In

addition, instead of looking exclusively at cross-sectional sexual dimorphism in callosal morphology, it may also be useful to examine the whether there are gender differences in CC development or vulnerability to pathological changes, or whether these are influenced by sex hormones.

3.1.2 Handedness Differences in the Corpus Callosum

While still uncertain, when compared to the findings in the gender difference literature, results seem to be in closer agreement when it comes to evaluating handedness effects on the CC. Witelson [50] found the corpus callosum to be larger in left-handed individuals (LH), with a significantly increased number of fibers present compared to right-handers (RH). While several studies confirm this finding [52, 53, 57], others fail to find any relationship between callosal morphological differences and handedness [33, 54, 55]. It seems that gender differences in conjunction with handedness differences may be co-related. It has also been found that hand preference was associated with CCA in men but not in women, with the isthmus appearing as one of the largest areas of disparity [50, 56]. Specifically, RH CCA was found to be smaller than non RH CCA (including LH and ambi/mixed-dexterity). However, a recent addition to the literature examined handedness and CCA in a large normative population sample, finding no relationships between handedness, total CCA or CCA of the anterior, middle and posterior thirds of the CC [48].

DTI has reported anisotropy differences related to a gender by handedness interaction. Westerhausen et al. [57] reported increased anisotropy in LH as compared to RH subjects, and in men as compared to women. In addition, they found significantly lower diffusion values in LH than RH subjects [57]. As a preliminary interpretation, these findings can be considered to show underlying microstructural differences between subjects

determined by gender and handedness. However, there have been a number of DTI studies which have found no relationship in similar conditions [57]. To interpret these differences, some researchers consider these gender specific handedness related changes to be a result of cerebral lateralization [32]. It is posited that if processing is confined to a single hemisphere, there would be a lesser need for interhemispheric transmissions, resulting in a decreased CCA. On the other hand, a pre-existing smaller CCA may result in more lateralized brain function [32].

Considering CC research more broadly, judging gender or handedness as critical variables within any population may be oversimplifying the matter. A number of studies have identified significant correlations of CCA with experience related phenomena [58]. It may be that factors such as occupation, language, physical activities and other experiences have salient effects. Normative populations cannot be simply classified as they appear on the surface, as no amount of screening questions would be able to identify all of the possible factors playing into callosal variability. With this in mind, an experience-dependent development of the CC is likely although it is unclear which and whether specific experiential demands (for example gender, i.e. society roles) are important as opposed to strict determination by biological sex. As a result, care must be taken when classifying and identifying sample populations. All of this talk of experiential importance cannot be considered without a concurrent discussion of heritability, as per the nature vs. nurture debate (i.e. epigenetic studies). Some twin studies have shown that CCA is under strong genetic influence [59, 60]. However, while this field begs further research, it is beyond the scope of the current discussion.

3.1.3 Age Differences in the Corpus Callosum

Also related to the discussion of gender and handedness is the consideration of age. The discussion about age related differences in the CC extends from the discussion of callosal development. As stated in an earlier section, it is unclear whether there is a significant age effect on CCA (see sections 2.1, 3.0.1). However, as identified in the DTI discussion, the DTI technique has shown effects of aging in terms of FA. Whichever the case, both the developmental and possible 'degenerative' courses of the CC would dictate that special attention be paid to subject groups and their matched controls. Groups of subjects aged below 30 years must be carefully considered, due to the possibility of possessing less 'stable' CCA (especially pediatric populations). Likewise, in elderly subjects care must be taken to ensure that other possible pathologies associated with aging (e.g. early dementia or high blood pressure) are taken into account. When interpreting results, it is important to consider that changes seen in advanced age may be related to factors such as neuronal cell death and resulting axonal elimination [50]. This may be particularly important for post-mortem studies, in which many subjects are of advanced age (or deceased as a result of other pathologies).

3.1.4 The Significance of Normative Differences

Despite the amount of research in normative populations, the persistence of inconsistencies indicates that the CC can be significantly different between individuals for a variety of reasons. The only firm conclusion that can be drawn is that there is no simple relationship existing between CCA and the discussed variables. In addition, these inconsistencies point to another factor – measurement error. It is possible that a number of normative differences exist, but their extent and nature cannot be clear without first eliminating methodological issues as a reason for the observed CCA variations. As such it

becomes important to examine the particular methods used in the segmentation of the CC, the focus of the following section.

3.2 Callosal Segmentation

3.2.0 Post-Mortem Segmentation

The earliest studies of the CC were performed in post-mortem brains, extracted after autopsy. It was a common procedure to recruit a sample of participants who were 'close to death' and perform psychological tests before death occurred. After death, brains were removed from the skull and perfused, fixing the brain [35, 50, 51]. To measure the CC, brains were bisected and the medial surfaces were exposed. These midsagittal slices were photographed with perpendicular rulers and traced, allowing measurements to be made [50, 51] (see Figure 3.2). These early studies sparked the discussion of handedness effects and sexual dimorphisms in the CC, preceding any studies involving neuropsychiatric disorders.

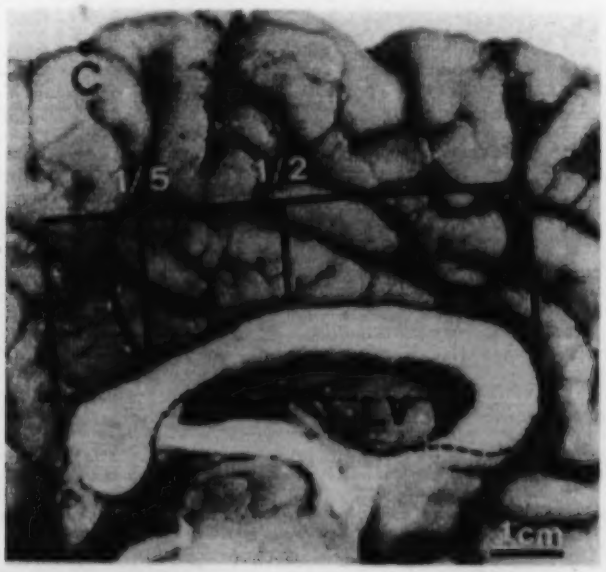

Figure 3.2: Divisions of the Corpus Callosum in Post-Mortem Studies (From Witelson, [50])

Post-mortem techniques, which provide excellent resolution when used with histological practices, cannot yet be matched by anatomical MRI when it comes to the detailed study of axonal anatomy [39]. This benefit is qualified by several confounding variables appearing in post-mortem brains. Factors include the possible deterioration of cellular materials (e.g. myelin) and changes in brain shape, size and chemistry caused by removal of the brain from the skull and fixation in formalin [38]. One example of this is swelling of certain regions due to absorption of CSF. Also needing consideration is the form that the brain takes when placed on a surface as opposed to an in vivo measurement and the resulting skewing of the 'natural' callosal shape. Many subjects included in post-mortem studies were deceased due to disease processes, old age, or traumatic events, all of which, along with any medical treatments, may have affected CC structure [39]. Lastly, post-mortem techniques are time consuming and require destruction of the brain material [5]. Although these studies have laid the groundwork for the field of callosal studies, these early findings need be considered with respect to their limitations. In their defense, comparative anatomical findings and nuclear magnetic resonance (NMR) techniques in post-mortem brains have not been found to show significant differences in measurements of corpus callosum surface area or overall brain volume [61]. However they may not be directly comparable with MRI studies.

3.2.1 Callosal Segmentation in Magnetic Resonance Imaging

Witelson [50, 51] proposed that CCA was a potential morphological marker for MRI to study normative and clinical populations. This statement has been put into practice, as numerous MRI studies have been conducted and for a substantial period it was the modality

of choice in CC characterization. While post-mortem studies present many potential confounds and only allow limited study of clinical characteristics, MRI involves less brain distortion and permits the implementation of concurrent clinical and psychological testing for relationships between morphology and behavioural measures of interhemispheric functions. Additionally, MRI data can be collected relatively quickly and in a form which can be readily modified, transformed and analyzed. To that end, Witelson [51] proposed a model which is one of the methods still used today to partition the CC. However, a number of other methods have arisen in an attempt to find the best way to segment the callosal regions. The subsequent section discusses several of these segmentation protocols which have been used in published CCA research and why they have contributed to inconsistencies in the literature.

The Straight Line Method

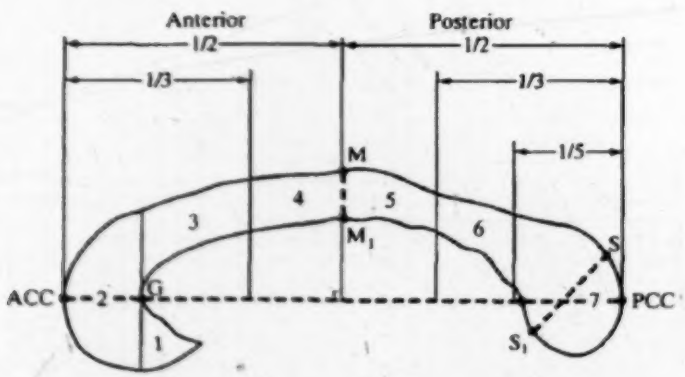

Figure 3.3: Witelson's Straight Line Model of Callosal Segmentation [51]

Outlined by Witelson [51], this schematic for subdividing the CC (Figure 3.3) has been adopted by some researchers to define the different regions of the CC. However, most researchers using this scheme have used an adaptation or their own interpretation of this

scheme to match their own protocol (likely a source of differences within the literature). The model outlines seven regions and corresponding defined anatomic labels. The name of the "straight line method" is derived from a line drawn from the most anterior to most caudal point of the CC (ACC-PCC line). This line is then divided into spaced sections. These sections correspond roughly to the divisions defined by fiber projections (refer to Table 3.0). The straight line model is advantageous as it clearly defines the different callosal regions. However, due to the limitation of MRI visualization of the CC as a homogeneous structure, it is nevertheless based on estimations rather than definite landmarks.

The Radial Method

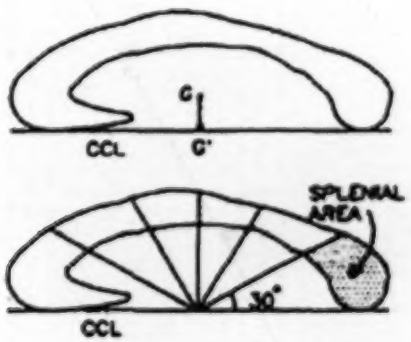

Figure 3.4: The Radial Method (From Bishop and Wahlsten, [39])

The radial method uses the idea of the 'centre of gravity' of the CC to create its divisions. The CC is first reoriented in the callosal plane, that is, where the inferior boundaries of the rostrum/genu and splenium of the CC are in the same horizontal plane (Figure 3.4). Using the callosal plane has the advantage of consistently orienting the CC without the need to use external landmarks for reference. When the centre of gravity is calculated, lines are drawn to cross the structure of the CC white matter at set angular

intervals. This method is easy to implement and compute, but lacks any real ability to separate the CC into specific regions by function, making it useful solely for general measurements and comparisons [29].

The Curved Line Method

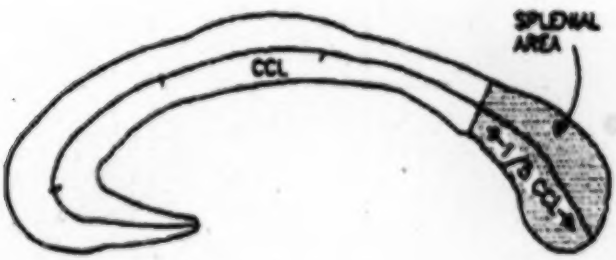

Figure 3.5: The Curved Line Method (From Bishop and Wahlsten [39])

The curved line method uses a continuous arcing line drawn between ventral and dorsal points through the CC, bisecting it lengthwise. Perpendiculars to this curved line are then drawn and the resulting sections produced are used to measure callosal volumes (see Figure 3.5). Rajapaske et al. [29] proposed a method of measurement based on the curved line method dividing the CC into 100 equally spaced sections by an automated procedure (see Figure 3.6). This technique is useful for computing volumes, but requires specialized computer software in order to create the divisions. Unfortunately, due to the arbitrary nature of these divisions as a whole, this method also does not divide the CC into consistent regions which are comparable across studies.

Figure 3.6: Modified Curved Line Method (Rajapakse et al. [29])

The Bent Line Method

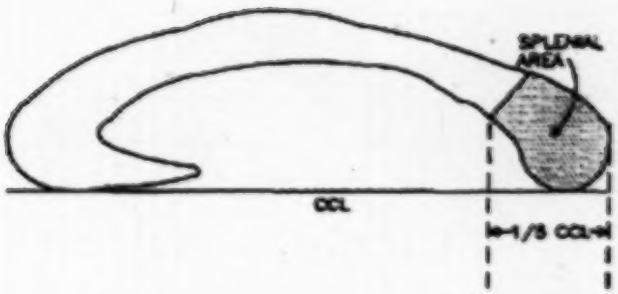

Figure 3.7: The Bent Line Method (From Bishop and Wahlsten [39])

The bent line method is most similar to the straight line method, as it is based on the establishment of an ACC-PCC line. The difference is that segments are drawn perpendicular to the wall of the CC at any given point, instead of the ACC-PCC line. This method is an alternative to the straight line method and does not seem to provide additional accuracy or precision when it comes to measurements [39].

Other Methods and Considerations

Other methods have been used to divide the CC into different parts, some dividing the CC into thirds [62] or fifths [40]. The splenium itself has been measured as the posterior

fourth, fifth or even tenth of the length of the CC [39]. These inconsistencies among measurement techniques, combined with the variations observed in normative populations make it clear that there are numerous factors which may be the root of differences between studies. Due to the small size of callosal regions, even a slight variation in measurement method (i.e. adaptations of the straight line method) can result in a significant difference in region measured, and ultimately in the final results [38].

3.3 Callosal Imaging

3.3.0 Image Acquisition

Researchers have developed a plethora of different MRI protocols and techniques in order to obtain accurate callosal measurements. Listing them all here would be excessive, but included in this section are some standard guidelines that have been proposed [38]. Specifically, MRI parameters like the specific pulse sequence, voxel size (3D volumetric unit of measurement), slice thickness and contiguity (i.e. with or without gaps between slices) are known to affect image acquisition, the signal-to-noise ratio and subsequently the quality of callosal measurements [38]. T1 weighting can be used when the CC needs to be visualized, as WM appears very bright while the surrounding GM and CSF appear dark.

Signal to Noise Ratio

Signal to noise ratio, or SNR, is defined as a measure of signal strength relative to background noise. If the noise level is too high, it can compete with the signal, making the image unreadable. As a result, SNR determines image quality and should be high to obtain good quality images and contrasts. In high quality images, callosal outlines are better defined

and measurement accuracy is increased. Bittoun et al. [63] state that, for a specific sequence, SNR is improved when the number of excitations and voxel size are increased. The drawback to increasing the number of excitations is that it requires longer scan times. As a result, the best parameter to manipulate is voxel size. Voxel size is determined by the field of view (FOV), matrix size and slice thickness. Studies have used FOV ranging from 180mm to 250mm, with the larger FOV allowing for a complete visualization of the CC and stronger measured signal intensity [38]. The FOV can be defined as the size of the spatial area of the image to be encoded [64]. The matrix size is a measure of the amount of data points collected and is inversely related to voxel size. Matrix size influences scan time and resolution- a reduced matrix increases voxel size and therefore SNR at the expense of spatial resolution. A matrix of 256 x 256 is commonly used in high resolution brain studies, but the size can be reduced to 128 x 128 or 64 x 64 in cases where subjects cannot stay still for long durations [64].

Pixel Size

Whereas a voxel is a three dimensional volume, a pixel is a two dimensional area. In determining the quality of slices, it is important that a small pixel size is maintained. With small pixel size, outlining of the CC is more accurate, as the WM, GM and CSF contrast will be clearly depicted. If pixels are large, partial volume averaging may occur, leading to inaccurate definition of callosal boundaries [38]. That is, in pixels larger than about $2mm^2$ an average of white and gray contained in the pixel will be displayed, giving the outline of the CC a rough appearance. High spatial resolution is also important to align the midsagittal slice, to ensure that measures are obtained close to this slice and because the CC is narrow.

In addition to small pixel size, it is also important that slices are thin to minimize partial voluming.

3.3.1 Image Processing

Following the acquisition of MRI images, they must be processed before data can be extracted. At this point, the CC image is prepared for measurement and different factors can be analyzed. Raw scanner data is converted into a specific file format which can be used by analysis software. Several research groups have developed specialized programs which are used to process images and allow data analysis of the brain in great detail. Each software suite has different capabilities and provides investigators with specific tools to assist in measurements of areas as well as other various capabilities. The most commonly used are Analyze, SPM, MINC Tools and NIH Image (individual specifications for these programs will not be discussed here). For visualization, it is important that software be capable of displaying simultaneous orthogonal sections- this is needed to identify the proper slices to be used in analysis.

In the image processing stage of callosal measurement, many variables come into play. With the aforementioned issues concerning individual callosal variabilities, it is important to standardize the analysis as much as possible. It is important to decide whether to compute measurements in 'native space' (absolute measurements), or 'stereotactic space' (after warping images to a common template) or both. Research has shown that values obtained by analyzing brains in different space can be significantly different- the approaches are not interchangeable or equivalent [65]. While analyzing brains in native space shows individual differences, allows comparison with existing literature and does not distort individual neuroanatomy, it requires additional control for differences in brain size, using

measures such as brain area, volume, or intracranial volume to normalize CCA, or as covariates in statistical analyses. Recent evidence has indicated that using different templates for stereotactic transformation of brain images can produce significant differences in CC proportional volumes that also differ from results using statistical correction for brain volume in native space [66].

In the processing stage it is important to screen out or correct image artefacts, including distortions due to displacement and skewing, which come as a result of subject movement or positioning during image acquisition, and intensity variation due to slice by slice variability in radiofrequency pulses. The next step is to reorient or in some cases discard images to ensure that the image orientation is appropriate for analysis. Although it is a requirement to orient images so that measurements are related to the MSS, some researchers have used the anterior commissure-posterior commissure (AC-PC) line [67] and some the callosal plane, but many have not indicated which reorientation method they have chosen. Others have simply not used subjects whose brains seem to be misaligned [38, 68]. In cases where brains are affected by lesions, they may also be omitted from the analysis.

The Midsagittal Slice Revisited:

In most studies, the MSS is manually selected using internal landmarks, for which brain orientation is important [33, 38]. Unfortunately, many researchers neglect to outline their landmarking protocols, which may account for some differences seen in the literature. Meisenzahl et al. [68] describes the 'hierarchical' landmarking process in detail-

> "First, slices were selected which showed no or only minimal white matter in the cortical mantle surrounding the CC. If more than one slice fulfilled this criterion, the

medial thalamic nuclei served as an anatomical landmark of a second order. The selected slice then showed the interthalamic adhesion connecting the left and right medial thalamic nuclei, or only the smallest size of the thalamus of either one or the other side. The transparent septum and cerebral aqueduct were used in the third step to confirm the selection when two slices remained which showed a similar amount of thalamic substance."

Research is emerging on the selection of the MSS through automated means and several techniques have been developed [69, 70]. Automated methods make use of a set of parameters derived from a 'training set' which contains certain landmarks and points of desired correspondence [70]. One method, Active Appearance Models (AAMs), uses these principles to establish object variability, and matches the rules and landmarks to find the desired slices. At present, the automated systems have not yet been demonstrated to be significantly more reliable than manual procedures, and are largely localized to the specific site developing the procedure.

Segmentation and Measurement

After the MSS slice has been selected, image contrast should be adjusted to clearly delineate the CC boundaries and remove unwanted GM or CSF from the analysis. In T1 scans, adjusting the contrast so that the GM in the image is very dark has the additional benefit of helping to deal with the issue of partial volumes as these pixels will be consistently eliminated. Automated methods are also available to segment brain structures into GM, WM and CSF. The next step is to define the CC. The most common way to delineate the CC has been to manually trace its borders. Manual tracing techniques can be time

consuming endeavours and are operator dependent, such that they require reliability tests [70]. Several different approaches have been used to manually trace the CC. Some researchers have captured images at the MSS and proceeded to obtain measurements from the CC image, much like obtaining measurements from post-mortem photographs [71]. Other researchers have made use of MRI data directly in real time with a brain image processing tool (one of the program suites referred to above). Although both techniques can obtain results, the use of the former may be prone to inaccuracy due to the conversion of matrix data to a simple image file, resulting in possible loss or distortion of data. Using data converted to images also presents difficulty in navigating through contiguous brain slices. The latter technique, while more powerful, requires that the researcher have training and experience to develop skill in the use of the brain processing tool.

Due to the discrete appearance of the CC at the MSS, automated methods of tracing have also been developed. Computer algorithms have been created to measure callosal areas, but as with other areas discussed, a single preferred automated segmentation method has not been used consistently throughout the literature [41, 72]. Templates have been used by several groups of researchers to compare morphological differences. Luders et al. [32] use a form of modeling by generating maps indicating callosal mean thickness (within groups) and comparing significant differences between groups. Dubb et al. [41] as well as Pettey and Gee [73] use a template matching method and template deformation morphometry. By using a male control as a template, a curve-registration algorithm is applied to detect regional size variations. As mentioned above, Stegmann and colleagues [70, 74] introduced the usage of AAMs for fully automated processing of the CC through the whole process (from selection of the MSS to CC boundary tracing). McInerney et al. [75] proposed a novel approach using 'deformable organisms'- autonomous computer programs that can

automatically segment, label and quantitatively analyze anatomical structures. These 'organisms' are perceptually aware of image analysis procedures and use sensed image features, pre-stored anatomical knowledge and deliberate plans programmed by the researcher to carry out their functions. McInerney et al. [75] demonstrated a CC worm (computer program) which was able to delineate the CC clearly in MSS MR images.

The final step in segmentation of the CC is measurement of the areas or volumes. For this function, automatic computer algorithms which count designated pixels are used. These pixels can be designated by a form of "brain painting", where the area of interest is manually selected by the investigator. In general, while manual techniques are more specific and detailed, automated techniques have the advantage of being able to process large samples efficiently and consistently. At this time, there is no research on which method is more reliable, and researchers often use a combination of manual and automated methods to arrive at their conclusions.

Conclusion

The study of the CC has yielded many interesting findings. Many different techniques have emerged which can be used to quantify callosal characteristics, but this has resulted in a double-edged sword; using such an array of methods means that each method is measuring something slightly different. It can be said that the field of CC measurement is confused and should be considered carefully in light of its intrinsic difficulties.

Chapter 4

The Corpus Callosum and Research in Depression

The identification of the underlying neural mechanisms of psychiatric symptoms is one of the main goals of modern psychiatry [6]. After the discovery of the inter-hemispheric integrative function of the CC, callosal abnormalities have been investigated in terms of their relationships to psychiatric disorders and the CC has been considered as a candidate region for pathology. As hemispheric specialization is important in aspects of sensory, motor and cognitive applications, functional deficits observed in psychiatric patients may be caused in part by callosal abnormalities. MRI and DTI techniques permit researchers to investigate potential abnormality of the CC at the structural level.

While researchers have studied many psychiatric disorders, the focus of this investigation centers on the mood disorder, major depression disorder (MDD). This chapter begins with a brief overview of MDD and continues on to discuss specific research pertaining to the callosal abnormalities with regards to MDD and disorders that show high comorbidity with MDD. Regional morphology is the basis of the research of most callosal studies but one of the accompanying caveats is that the size or configuration of any brain structure is not necessarily a direct reflection of its role in brain function [76].

4.0 What is Depression?

According to the World Health Organization [1], MDD is the most prevalent mental health problem in primary healthcare settings worldwide [77]. The WHO also estimates that

by the year 2020, depression will be the 2nd leading cause of disability for all ages and sexes. Today, it is already the 2nd leading cause of disability of people aged 15-44 and it occurs in all genders, ages and backgrounds [1].

MDD and bipolar disorder are the main mood disorders. Individuals affected by MDD experience episodes of persistent symptoms such as a pervasive low mood, loss of interest in usual activities and a diminished capacity to experience pleasure, together with characteristic vegetative and cognitive symptoms, e.g. changes in appetite, sleep, motor activity, concentration (see Appendix A1) [78]. These difficulties are sufficiently severe to adversely affect social and occupational functioning. In addition to MDD, variants and subtypes of depression include bipolar depression, dysthymia, atypical depression, post partum depression and seasonal affective disorder.

The cause of depression is unclear, but it is thought to be a combination of psychosocial and physiological factors. Genetic variation, adverse life experiences, stress hormone dysregulation, alterations in prefrontal and limbic brain structures, sex and gender, medical conditions and nutrition are among factors implicated in depression (see Appendix A2). Classical twin and adoption studies have confirmed modest heritability of MDD, suggesting an important role for genetic variation leading to disease pathology [79]. Molecular genetic studies have begun to suggest that genetic risk may interact with specific environmental risk factors, such as childhood maltreatment and/or adult stressors (e.g. Caspi et al. [80]).

Physiologically, the hypothalamic-pituitary-adrenal (HPA) axis is known to show altered function in depressed patients and may result in alterations in brain structure. In particular, the corticotrophin-releasing hormone circuits are thought to be overactive, resulting in enhanced levels of cortisol [81, 82]. Additionally, MDD has been linked with a

reduction of hippocampal volumes, with the magnitude of GM decrease being related to duration of illness [83]. Tentative explanations of the observed decreases in GM propose that neurotoxic effects of persistently elevated glucocorticoids may adversely affect hippocampal neurogenesis [81]. However, it is equally possible that synaptodendritic, axonal, or glial components are altered [84, 90].

While a few studies have not revealed significant differences [85, 86], the majority of research has implicated alterations in prefrontal cortex in depressive disorders [87]. A recent review of 140 structural MRI studies [88] indicates that while whole brain volumes of mood disorder patients are not significantly different from healthy controls, prefrontal deficits- especially in the anterior cingulate and orbitofrontal cortex- are consistently observed. A quantitative meta-analysis indicated that the anterior cingulate cortex below the genu of the CC shows a significant reduction in volume [89]. Reductions in the dorsolateral prefrontal cortex and dorsomedial prefrontal cortex have been demonstrated [90]. Other findings to date also suggest that subcortical structures such as the striatum and amygdala, in addition to the hippocampus, are affected [88].

Notable to the current discussion is the presence of gender differences in the prevalence of MDD. Rate differences appear as early as adolescence, with girls showing increased rates of depressive symptoms or disorders while boys show little or no increase [91]. A number of studies have investigated the basis or these differences, tentatively identifying several factors which may account for the greater percentages of women diagnosed with MDD. As with MDD, the sex disparity is likely a result of an interaction between a number of different factors. These factors include negative life events, negative cognitive style (individuals who attribute negative life events to global, stable causes or have

dysfunctional attitudes about the world), genetic vulnerability, HPA axis dysregulation, and hormonal changes [91].

Life stressors have long been identified as risk factors for depression (see Appendix A2) and it has been proposed that the gender disparity is closely related to the differences between the way men and women are exposed to and respond to these life stressors [92]. A psychosocial aspect may also be involved as while men report difficulty coping with occupational and financial problems, women identify issues such as relationship problems, illness/loss of individuals in their distal network and lack of adequate housing as stressful life events [91]. Women are also more often victims of gender discrimination.

In particular, some stressors affect women more severely, with women at a far greater risk of physical and sexual abuse. Severe stress early in life is thought to cause sensitization of the autonomic stress response and the HPA axis, resulting in the increased risk of adult pathological disorders. Some studies have shown that increased pituitary reactivity to stress may be a biological vulnerability for the development of stress-related disorders [93]. It is suggested that the hyperreactivity of the stress response system is a persistent consequence of childhood maltreatment.

Hormonal changes are often thought to be important in the MDD gender difference as depression becomes most prevalent in early adulthood, with women of childbearing age being particularly susceptible to depression. The perimenopausal transition is also a risk factor for depression [91].

4.1 Literature on the Corpus Callosum in Depression

Data from a variety of disciplines has suggested that there is some sort of cerebral lateralization in depression, implying that interhemispheric information transfer may be

affected [24]. Following the Witelson Nowakowski Principle [94] greater degrees of asymmetry are associated with fewer interhemispheric connections, implying that a change might be seen in commissural tissues such as the CC. The involvement of frontal regions, especially in areas of the cingulate cortex, suggests that the frontal CC should be investigated for any detectable abnormalities. In addition, two case reports have described patients with anteriorly and medially placed CC tumors who presented primarily with depressive symptoms [98, 99].

The literature on CCA in MDD is relatively sparse, for example compared with studies of the hippocampus. The few studies of the CC that have been conducted with depressed patients have reported somewhat inconsistent results, which are summarized in Table 4.0. The most consistent finding to date has been that studies have not shown differences between depressed subjects and controls in total CCA. Two studies did not examine CC subregions [96, 97], which have been measured in only four publications. Wu et al. [95] reported larger anterior and posterior quadrants of the CC in MDD patients. Lacerda et al. [24] found no differences from controls within their MDD sample as a whole. They did report significantly increased areas of the genu, anterior midbody and middle splenium (using an adapted version of Witelson's scheme) in MDD patients with a positive family history in first degree relatives, when compared with both non-familial depressed patients and control groups. This should be seen as an exploratory finding, given that a larger number of subdivisions were examined than in the basic Witelson scheme and that the subgroups of MDD subjects were small. In a 2002 study, Lyoo et al. [71] found a significantly smaller genu and posterior midbody volumes (following the Witelson scheme) in a sample of young women who met diagnostic criteria for early-onset dysthymia and/or depressive personality disorder, rather than MDD [71].

The inconsistencies between studies may in part be related to factors discussed in the previous chapter on callosal measurement, with the acquisition of relatively thick slices (3.0 – 5.0 mm) in four studies and variations in the segmentation protocol, but differences in patient sample characteristics may also be relevant. One factor is age, since although it was earlier stated that the CC is relatively stable in normative populations through the adult life, in the case of MDD, factors such as age of onset, disease chronicity, severity, medications and treatment duration may be important considerations. The mean ages of the subjects in the Wu et al. [95] and the Lacerda et al. [24] studies were similar, whereas subjects in the studies by Husain et al. [96] and Parashos et al. [97] had a mean age in the 50s and the range extended to subjects with advanced age (up to 80 years). In contrast Lyoo et al. [71] included only young subjects, with a mean age of 21.4 years. Only Lyoo et al. [71] examined a sexually homogeneous population controlled for handedness. Although some studies reported the exclusion of subjects with recent alcohol or substance abuse/dependence, lifetime history was not controlled.

The fourth publication on CC subregions was a recent study of late life depression by Ballmaier and colleagues [100] that addressed callosal subregion areas (using Witelson's scheme) and callosal thinning in relation to age of onset. It was reported that a reduced area and thinning of the anterior genu was observed in both early-onset ($\leq$ 60 years) and late onset MDD, whereas only patients with late-onset depression showed a reduced area and thinning of the splenium. Additionally, measures of memory and attention correlated with genual and splenial thinning in late-onset MDD.

Despite the equivocal results in terms of the direction of change, these findings suggest that the anterior portions of the CC (anterior quarter or third, genu, rostrum) may show alterations associated with depression in young adults. This would be consistent with

evidence for prefrontal changes in MDD, discussed above. Posterior changes in the CC have also been noted, however, there are only two volumetric studies of temporal lobe volume in MDD and none of parietal and occipital lobe volumes [88].

There have been very few DTI studies of the CC in MDD populations. Two recent studies have examined white matter in late-life depression. Nobuhara et al. [101] found only an insignificant decrease in FA within the CC in depressed patients compared with healthy controls, but found significant reductions in frontal and temporal lobe WM. Similarly, Yang et al. [102] found no FA reduction in the CC itself, but significant reductions in the superior and middle frontal gyri as well as the right parahippocampal gyrus. Due to the fact that both of these studies examine late-onset depression, these results cannot necessarily be generalized to all depressed populations.

Study	Subjects	Age (y) Range or mean ± SD	Sex % F	Trauma	Slice orientation, thickness (mm)	In-plane resolution (mm)	CCA	CCA Subdivision	Brain size correction
Husain et al. 1991 [96]	20 MDD 20 Con	23 - 80	75 75	NR	Sagittal, 3.0 - 5.0	NR	=	NR	Brain area ratio
Wu et al. 1993 [95]	20 MDD 16 Con	33 ± 12 31 ± 10	55 38	NR	Sagittal, 5.0	3.0 x 3.0	=	Anterior ¼, ↑ Posterior ¼, ↑	Brain area ratio
Parashos et al. 1998 [97]	32 MDD 32 Con	55 ± 17 55 ± 17	63 58	NR	Axial, 5.0	NR	=	NR	Brain area ratio
Lyoo et al. 2002 [71]	40 DD 42 Con	21 ± 2 21 ± 3	100 100	†NR	Coronal, 1.5	1.5 x 1.5	=	Genu ↓ PMB ↓	Brain volume covariate
Lacerda et al. 2005 [24]	22 MDD 39 Con	41 ± 11 36 ± 11	86 38	NR	Sagittal, 3.0	0.9 x 1.3	=	= in MDD *Anterior ⅓ ↑ *Splenium ↑	ICV covariate
Ballmaier et al. 2008 [100]	46 MDD 34 Con	71 ± 7 72 ± 7	74 56	NR	Coronal, 1.4	0.9 x 0.9	NR	Anterior ⅓ ↓ ‡Splenium ↓	Brain volume Covariate

Table 4.0: Summary of Results from Prior Studies of the Corpus Callosum in Depressive Disorders

Abbreviations: MDD = Major Depressive Disorder, DD = Dysthymic Disorder, Con = control, NR = not reported, anterior ⅓ = rostrum + genu + rostral body, PMB = posterior midbody, Posterior ¼, ↑ ↓ decrease, ↑ increase, = no difference, ICV = intracranial volume.

* ↑ only in MDD subjects with family history of MDD in first degree relatives.

†Childhood maltreatment not quantified, but population reported clinically to have frequent maltreatment.

‡Reduction in splenium limited to MDD with late onset (after age 60 years). Callosal thickness also reduced in genu and splenium

4.2 Childhood Maltreatment, MDD and the Corpus Callosum

Childhood maltreatment, also designated as childhood interpersonal trauma (CIT), can be divided into several different subgroups on the basis of the type of maltreatment involved. These groups have been identified by qualitative research, but quantitative research is only just beginning to take them into consideration [27]. As a result, scientific research remains fragmented and significant gaps in the knowledge base need to be filled [103]. Childhood sexual abuse (CSA) involves sexual contact, violation of privacy or inappropriate exposure of a child to adult sexuality [103, 105]. Child physical abuse (CPA) is defined as intentional injury inflicted on children and can take the form of striking, burning, shaking or other actions causing physical harm. Although CSA and CPA have received the most attention, the remaining facets of CIT, emotional abuse and neglect, are thought to be the most common. Emotional abuse can include verbal abuse, withholding of affection, invalidation of feelings and witnessed violence. Neglect is associated with physical, educational and emotional aspects. Physical neglect involves the failure to provide a child with basic needs and health care, and disregard for his or her safety. Educational neglect can include failure to enroll a child in school and lack of concern for the educational needs of a child. Emotional neglect is related to emotional abuse and can include inadequacies in nurturing or affection, exposure of a child to spousal abuse, failure to intervene when a child exhibits antisocial behaviour, and refusal or delay in providing psychological care [104]. It should be recognized that these groups are not mutually exclusive and can often coexist. A significant amount of child abuse has been identified as being committed by family members or those close to the victim [106], resulting in issues related to disclosure and its consequences [105].

In addition to potential reasons for inconsistency in CC findings in MDD discussed above, another factor may be heterogeneity within the diagnostic category. One example noted above was that direct comparison between controls and early- and late-onset MDD subjects showed that only the last group had significant splenial changes [100]. The present thesis addresses childhood maltreatment as factor that might potentially contribute to heterogeneity in studies of MDD in younger adults (< 50 years). Childhood maltreatment is an important factor to consider because it is an established risk factor for the subsequent development of MDD, may contribute to the sex difference in the prevalence of MDD, as described above, and because it has been associated with changes in CCA in several studies in populations where MDD was a frequent secondary diagnosis. The next section expands on these studies, which are summarized in Table 4.1.

The CC and CIT

In a study of pediatric patients admitted for psychiatric evaluation, half of whom had histories of CIT, Teicher et al. [27] found a 17% decrease in CCA in CIT-exposed patients compared with healthy control subjects. The CIT-exposed patients also showed an 11% decrease in CCA when compared to the remaining non-exposed patients admitted for psychiatric evaluations. Specifically, the anterior and posterior midbodies, as well as the splenium showed significant reductions in area.

Findings in PTSD related to CIT are also relevant to depression, because of the frequent comorbidity of depressive disorders with PTSD, because both disorders involve alterations in HPA axis function, and because there is substantial overlap in terms of volumetric changes on MRI. A recent meta-analysis has shown that PTSD is associated with reduced hippocampal, anterior cingulate cortex and prefrontal volumes [107]. PTSD has

only been examined recently with regards to callosal morphology, but two studies of pediatric samples have shown a smaller total CCA in PTSD associated with CIT [108, 109], which were summarized in a pooled analysis of a large sample [110]. This analysis showed alterations in all CC subregions except the rostral body and rostrum, but the rostrum showed a significant diagnosis x age interaction, in that PTSD subjects did not show the normal age-related increase in rostrum area that was seen in healthy controls. Notably, the majority of PTSD subjects in this sample had comorbid MDD or dysthymic disorder. In an adult sample, half of whom had experienced CIT and all with a lifetime history of MDD, decreased total CCA and decreased genu, anterior midbody, posterior midbody and isthmus subregions have also been demonstrated [111]. In a further adult sample, all of whom were exposed to CIT and the majority of whom had comorbid MDD, Kitayama et al. [112] found no differences in total CCA or the absolute area of individual subregions, but the subregion to total CCA ratio was significantly decreased in the posterior midbody when compared to healthy controls. A recent DTI study [113] also reported decreased FA of the anterior and posterior midbodies in a pediatric sample (mean age, 10.6 years), exposed to CIT.

Borderline personality disorder (BPD) is also frequently associated with CIT and adult diagnoses of MDD and PTSD, and with co-morbid attention-deficit hyperactivity disorder (ADHD). In a study of 20 women with BPD with comorbid ADHD and matched controls, Rusch et al. [114] found that BPD patients, fourteen of whom also had comorbid MDD, showed a smaller posterior midbody area and thinner isthmus of the CC when compared with healthy controls. Moreover, women with BPD and a history of childhood sexual abuse presented with a smaller and thinner posterior midbody and isthmus than BPD subjects without a history of childhood sexual abuse.

Conclusion

The above findings suggest that childhood maltreatment may be associated with reductions in total CCA and in CC subregion areas in pediatric and adult samples with diagnoses associated with frequent depressive comorbidity. These studies have quite consistently shown alterations in the posterior midbody and frequently alterations more posteriorly and in the anterior portion of the CC, without finding effects on the rostral body. However, to date, CIT has not been examined as a potential factor in studies of adults with a primary diagnosis of MDD.

Study	*Subjects	Age (y) Range or mean ± SD	Sex % F	Trauma	Slice orientation, thickness (mm)	In-plane Resolution (mm)	CCA	CCA Subdivision	Brain size correction
De Bellis et al. 2003 [110]	61 PTSD (31 +MDD) (41 +DD)	12 ± 3	51	CIT	Coronal, 1.5	0.9 x 0.9	↓	†Rostrum = Genu ↓ AMB ↓ PMB ↓ Isthmus ↓ †Splenium ↓	ICV covariate
	122 Con	12 ± 3	51						
Teicher et al. 2004 [27]	41 Psych	13 ± 3	54	28 CIT	Sagittal, 1.5	0.9 x 0.9	↓	AMB ↓ PMB ↓ Splenium ↓	Brain area covariate
		13 ± 3	43	23 No					
	115 Con	12 ± 4	39	No					
Villareal et al. 2004 [111]	12 PTSD (12 +MDD)	43 ± 9	80	6 CIT 6 Adult	Coronal, 1.5	0.9 x 0.9	↓	Genu ↓ AMB ↓ PMB ↓ Isthmus ↓	Brain volume ratio
	10 Con	44 ± 11	75						
Kitayama et al. 2007 [112]	9 PTSD (8 +MDD)	38 ± 10	100	CIT	Axial,		=	PMB ↓	Total CC area ratio
	9 Con	37 ± 9	100						
Rusch et al. 2007 [114]	20 BPD (14 +MDD)	26 ± 6	100	10 CSA	Coronal, 1.0	1.0 x 1.0	NR	‡Anterior ⅓ = ‡PMB ↓ ‡Isthmus =	Brain volume not different
	20 Con	27 ± 8	100						

Table 4.1: Summary of Results from Prior Studies of the Corpus Callosum in Disorders Related to Childhood Maltreatment

PTSD = Posttraumatic Stress Disorder, MDD = Major Depressive Disorder, DD = Dysthymic Disorder, BPD= Borderline Personality Disorder, Con = control, NR = not reported, AMB = anterior midbody, PMB = posterior midbody, anterior ⅓ = rostrum + genu + rostral body, ↓ decrease, ↑ increase, = no difference, ICV = intracranial volume

*Parentheses refer to number of subjects with comorbid disorder, +MDD, +DD or +PTSD).
† Diagnosis x age interactions were present, with rostrum and splenium failing to show normal age-related increases in area.
‡Callosal thickness reduced in posterior midbody and isthmus, whereas tended to increase in rostrum and genu, particularly in borderline subjects with CSA.

Chapter 5

Research Question and Hypotheses

Reports about callosal morphology in MDD patients have been somewhat inconsistent. Whereas no studies have reported changes in total CCA, several studies have reported changes in anterior portions and posterior changes have also been reported. Exposure to CIT is one factor that might contribute to heterogeneity in studies of the CC in MDD, since CIT has been consistently associated with changes in total CCA and CC subregion areas in samples with frequent depressive comorbidity. As CIT is a risk factor for MDD and several other psychiatric disorders, and adverse experiences and associated stress could potentially be a cause of altered brain and CC development, study of the contribution of CIT may add to understanding of CC morphology in MDD.

Within the same adult MDD and control sample on which this thesis is based, a DTI examination showed that patients with MDD had reduced FA in WM tracts that pass through the genu and rostrum of the CC, projecting to medial prefrontal cortex (Coupland, unpublished data). However, this alteration was present only in subjects who had experienced childhood sexual and/or physical abuse. Analysis of ventromedial prefrontal grey matter (vmPFC) volume in this sample also showed a trend ($p = .08$) to reduced vmPFC volume in MDD subjects with a history of childhood sexual and/or physical abuse and a significant reduction ($p = .02$) in vmPFC volume in MDD subjects who had experienced both childhood sexual/physical abuse and emotional abuse or neglect. These findings, which are also described in Appendix C for clarity, further suggest that there might

be alterations in CC subregion areas in MDD in association with CIT, particularly in the genu and in the rostrum, which has projections to vmPFC.

From literature described in the previous sections, several hypotheses can be proposed regarding the CC in MDD. **The first is that changes in total CCA may not be associated with MDD per se, but may be evident in MDD subjects with a history of CIT. The major hypothesis of the study is that MDD with a history of CIT would be associated with anterior CC changes, affecting the rostrum and/or genu, rather than the rostral body. Finally, in the posterior CC, MDD associated with CIT would most strongly be predicted to show changes in the posterior midbody.**

The studies referred to have addressed CIT in both pediatric and adult populations with differing primary diagnoses. Although the pediatric studies of CIT show reductions in CC areas, it is not certain that this would necessarily continue into follow up, since later development may include pruning of axonal connections [115]. In addition, it has been inconsistent in prior studies of MDD whether CC subregions have been decreased or increased in area. These uncertainties and the limited study of the topic to date indicate that hypotheses should be two –tailed, rather than unidirectional.

Chapter 6

The Study

6.0 Methods

6.0.1 Participants:

Participants gave fully informed written consent to take part in the study, which was approved by the Health Research Ethics Board of the Faculty of Medicine and Dentistry, University of Alberta. MDD and control subjects were included into the study according to the following criteria:

Inclusion Criteria

All participants were right handed, based on the Edinburgh Handedness Inventory laterality index $\geq +80$ [116].

In total, 38 subjects aged 21 – 50 years meeting DSM-IV criteria MDD of at least moderate severity were included, based on the Structured Clinical Interview for DSM-IV (SCID) [117].

Controls (n = 34) were included who were matched as a group for age, sex, years of education and smoking.

Exclusion criteria

(1) In order to reduce heterogeneity within the MDD sample, subjects were excluded if they had a history only of mild MDD, MDD with atypical features, a purely seasonal affective disorder, or MDD with psychotic features.

(2) Lifetime schizophrenia, bipolar disorder, alcohol/substance dependence, anorexia nervosa, antisocial or borderline personality disorder (DSM-IV).

(3) Lifetime alcohol/substance abuse lasting greater than 12 months, or occurring within 12 months of scans.

(4) Significant medical disorders that might influence brain structure, for example brain injury, epilepsy, hypertension, diabetes, or patients treated with systemic corticosteroids.

(5) Marked obesity (for practical reasons).

(6) Pregnancy or lactation.

(7) Contra-indications to magnetic resonance scanning, including presence of metallic objects or medical devices.

Recruitment and Assessment

MDD and control subjects were recruited concurrently via notices in community, primary care and hospital settings and were assessed in the outpatient psychiatry department. Recruitment materials for MDD targeted depression and not childhood maltreatment. Diagnoses were assigned following a clinical psychiatric assessment and SCID interview by an experienced researcher trained in the use of the SCID (Dr. Nick Coupland).

CIT Subgroups

CIT was rated using the Childhood Trauma Questionnaire [118]. The CTQ is a 28-item self-report inventory that provides a brief, reliable and valid screen for histories of childhood abuse and neglect. The scale includes 5 subscales, physical abuse, sexual abuse,

emotional abuse, emotional neglect and physical neglect, to follow common definitions of childhood maltreatment. Each subscale is scored based on 5 items and an additional 3 items are scored to rate denial or minimization of symptoms. The items are designed to elicit general memories of the frequency of being treated in certain ways, rather than the specifics of time, place or person, or the respondents' interpretations of whether the experiences were abusive. Items are scored on 5-point Likert scales according to frequency (never true, rarely true, sometimes true, often true, very often true). The separation into five subscales has been supported by confirmatory factor analysis in three independent samples [118]. The subscale scores have shown high internal consistency (Cronbach alpha > .80) across multiple samples, with the exception of the physical neglect scale (typically .60 - .70). The subscale scores have good convergent validity with therapist ratings of CIT and other scales.

CTQ scores are not generally normally distributed and were positively skewed in the present study. Subjects were therefore categorized into subgroups for analysis, based on cut-scores. Standard cut-scores according to the CTQ manual were used to identify subjects with moderate-severe sexual abuse (≥8), physical abuse (≥10), emotional abuse (≥13) or emotional neglect (≥15) [118]. These cut-scores have also been corroborated against experienced therapists' independent ratings of maltreatment judged to be clinically significant. Physical neglect was not used as a category, because of the lower consistency and convergent validity of this subscale, severity was low and it did not occur independently of emotional abuse and neglect.

In order to minimize the number of categories and analyses, MDD subjects were classified as having childhood sexual/physical abuse if they met the cut-score for either (n = 18). Although it might be anticipated that these subjects would have more frequent emotional abuse and neglect, this was not the case. Emotional abuse and neglect were highly

inter-correlated (rho = .61; $p < .0001$), but the correlations of emotional abuse with sexual abuse (rho = .30; p = .07) or physical abuse (rho = .19; p = .26), or of emotional neglect with sexual abuse (rho = .18; p = .28) or physical abuse (rho = .08; p = .65) were not as closely related. MDD subjects were therefore classified as having childhood emotional abuse/neglect if they met the cut-score for either (n = 21).

Medicated and Unmedicated MDD Subgroups

Previous studies have not always been explicit regarding the recent treatment history of MDD subjects. Even where explicit about current medication, treatment and washout durations may have been very brief, for example, two weeks. In the present study, MDD subjects were only included if they had been receiving long term antidepressant medication or had not been receiving medication for a substantial period. 16 *medicated* MDD subjects had received continuous antidepressant treatment at recommended dosages for ≥6 months (median 36, range 7 - 144 months). None were receiving lithium or anticonvulsants. 22 *unmedicated* MDD subjects had not received a course of antidepressant treatment within 6 months. 11 were antidepressant naïve, 11 had a median 24 months washout (range 6 - 168 months).

Other Assessments

Current symptoms were rated using the Hamilton Depression Rating Scale, a 17-item clinician-rated assessment of depressive symptoms [119, 120], and the Mood and Anxiety Symptoms Questionnaire – short form, a 64-item assessment of anhedonia, negative affect and somatic anxiety symptoms [121]. Patients were also assessed for whether they had multiple depressive episodes (≥ 3, because the overall duration of depression was similar in

subjects with 1-2 episodes, but longer in subjects with more episodes), whether they had ever made suicide attempts and whether they had experienced psychological trauma in adult years.

6.0.1 MR Image Acquisition

T1-weighted 3-D Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) images were acquired at 1.5 T (Siemens Sonata, Siemens Medical Systems, South Iselin). Head motion was restricted using memory foam and bilateral head clamps were used to support cushioned earphones. The scan parameters were: repetition time, TR = 1800ms; echo time, TE = 3.82ms; inversion time, TI = 1100ms; flip angle = 15°; field of view, FOV = 256 x 256 mm; matrix = 256 x 256; slice thickness = 1.5mm, no gap; 128 coronal slices. The acquisition was orientated perpendicular to AC-PC line based on TurboFlash localizer images (ultra fast gradient echo pulse sequence).

6.0.2 MR Image Processing

The Montreal Neurological Institute (MNI, Montreal, Quebec, Canada) suite of brain imaging tools was used for image processing. After acquisition of raw data, images were converted to the MINC file format, which was used throughout the analysis.

The first processing step was intensity non-uniformity correction using the N3 tool which corrects artifacts seen in MR data due to such factors as poor radio frequency (RF) field uniformity, eddy currents driven by the switching of field gradients, and patient anatomy both inside and outside the field of view. The next step was to examine the brain images using the MNI program Register to determine the magnitude of pitch, roll and yaw rotations. After these values were determined, the Mincresample tool, which resamples files

along a new spatial dimension with new voxel positions as specified by the user, was used to reorient the brain to the callosal plane and midsagittal slice, according to the angles of rotation determined in Register. In this step, the voxels were also resampled to 0.5 x 0.5 x 0.5 mm^3 dimensions. The midsagittal slice was identified according to the criteria of Meisenzahl et al. [68].

The regions of interest (ROI, in this case, the CC and its subregions) were then segmented using the Display software, which allows simultaneous visualization of the three orthogonal planes. This program was also used for the volumetric measurements of the regions of interest. The segmentation protocol is described in detail in the following section. Additional documentation about MINC Tools can be found on the Montreal Neurological Institute website [122].

6.0.3 Segmentation

Contrast Adjustment

In order to better define the borders between GM, WM and CSF, contrast adjustment was necessary. Images were set to grayscale. Contrast adjustment was performed at the level of the MSS. As the CC is a discrete structure (it connects the left and right hemispheres at the midsagittal slice without being bordered by GM) it is possible to achieve a fine contrast between CSF and WM. To achieve the clearest possible boundaries, first the gray/white threshold was adjusted so that only a few gray inclusions appeared in the corpus callosum. The gray/CSF threshold was adjusted so that GM was very dark, almost as to not be visible. After this contrast was established, it was used across all files. If necessary, minimal adjustments were made to optimize the images. Partial volumes were considered to be gray pixels adjacent to WM pixels connected to the corpus callosum.

Tracing and Volumes

Although automated methods of segmenting the CC are available, it was decided that manual delineation would be used in this investigation. Manual delineation allows more accurate judgment of specific detail than model or matching templates and when measuring small areas such as the sub-regions of the CC, precision is a priority. Furthermore, due to the prevalence of individual differences seen in the CC, when examining small regions, a human employing a set of rules may be better able to adapt as compared to model or template based segmentation methods.

The Display suite has an interactive paintbrush/fill tool which was used to trace ROIs. In addition, a threshold was set on the tool so that it discriminated between pixels of certain intensity. As the difference in intensity between callosal WM and CSF is quite stark, after being adjusted for contrast, this thresholding property was useful to trace the structures of interest (as well as exclude partial volumes). Since at the MSS the CC is free from GM connections, partial volumes were considered to be callosal WM.

In order to minimize measurement error, the MSS and two adjacent slices were traced for each ROI in each MRI image, and the resulting volumes averaged. In other words, the sagittal slices directly adjacent (lateral, left and right) to the MSS were designated as the other midsagittal slices. This allowed multiple CC measures to be obtained for each subject, but since the slices were thin (0.5 mm after resampling), all measures were acquired from the midsagittal 1.5mm of the CC. After ROIs were traced and filled, total filled voxels were calculated automatically. Each brain region was painted with a different label to allow for clear and efficient segmentation (see Figure 6.1).

CC Tracing

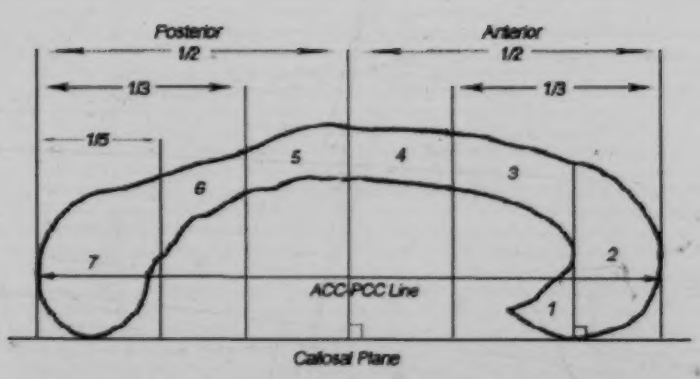

Figure 6.0: Current Segmentation Model of the Corpus Callosum

Figure 6.1: Sagittal MR Image of Manual Segmentation of the Corpus Callosum in Display

The CC was segmented according to a slightly modified version of Witelson's [51] model (see Figure 6.0). Instead of the model as originally defined (see Figure 3.3), the callosal plane was used to define the genu and rostrum, as it provided the most consistent plane across CCs of different shape. The ACC-PCC line was also determined for other measures. The analysis was performed by a single investigator (JF) blind to the diagnostic and clinical information of the study subjects. Intra-rater reliability tests were performed at an interval of 60 days. Eight measurements were calculated – total CCA as well as the area of the 7 callosal subdivisions (Rostrum, Genu, Rostral Body, Anterior Midbody, Posterior Midbody, Isthmus, and Splenium). A specific delineation protocol was established for each region, as described below, and the methods were repeated uniformly for all subjects.

Total CCA

Using a brush size of a single pixel, the outline of the CC at the MSS was traced. After borders were established, the interior was filled and volume automatically calculated using the Display volume calculation function (mm^3). After the CCA of the MSS was recorded, the process was repeated for the slices adjacent to the MSS (to the left and right). The three volumes were then averaged.

Genu Tracing

In order to trace the boundary of the genu, the midsagittal plane was examined along with the coronal plane. The most posterior coronal slice where the corpus callosum appeared as a single discreet slice with no break was judged to be the posterior boundary of the genu. All of the callosal area for the midsagittal slice including and anterior to this coronal slice was judged to be the genu. This area was first traced with a single-pixel cursor

and then filled using the thresholded paint tool. As for total CCA, the midsagittal slice and the two lateral midsagittal slices were computed for volume and averaged.

Figure 6.2: Segmentation of the Genu of the CC

Rostrum Tracing

As with locating the boundary of the genu, determining the boundary of the rostrum was identified by finding the slice just posterior to the slice described above. In other words, the most anterior coronal slice in which the corpus callosum does not appear as a single discreet structure is designated as the anterior boundary of the rostrum. This slice and all posterior slices with rostral white matter were designated as the rostrum. The tracing and volumetric computation was as per technique mentioned for genu tracing above. For both genu and rostrum tracing, if there was any question as to the 'connectivity' or 'discreetness', the genu is defined to be the white matter anterior to partial volume pixels.

Figure 6.3: Segmentation of the Rostrum of the CC

While specific rules for segmentation were necessary for the rostrum and genu, for the remaining regions, sub-region definition followed Witelson [51] in using ratios to the length of the ACC-PCC line. Using the coordinates given by cursor placement in Display, determination of the ACC-PCC line was carried out for all subjects. The cursor was placed at the foremost point of the genu and the most posterior point of the splenium on the MSS. The x distance was calculated and used to measure out the remaining subregions according to the specification of the segmentation model.

Rostral Body Tracing

The anterior boundary of the rostral body was designated as the previously established posterior boundary of the genu, while its posterior boundary is designated as 1/3 of the ACC-PCC. Tracing and volume measurement was carried out in a similar manner to the above.

Anterior Midbody Tracing

The anterior boundary of the anterior midbody was the anterior 1/3 of the ACC-PCC line, while the posterior boundary was set at the ½ mark. Tracing and volumetric measurement was as above.

Posterior Midbody Tracing

The anterior boundary of the posterior midbody was the ½ way mark of the ACC-PCC line. The posterior boundary of the posterior midbody was set at the posterior 1/3 ACC-PCC mark. Tracing and volumetric measurement was as above.

Isthmus Tracing

The isthmus was defined as the section between the posterior 1/3 ACC-PCC mark and the posterior 1/5 ACC-PCC mark. Tracing and volumetric measurement was as above.

Splenium Tracing

The splenium was designated as the posterior 1/5th of the CC, as determined by the ACC-PCC distance. Tracing and volumetric measurement was as above.

Intracranial Volume (ICV)

ICV was measured using the method of Eritaia et al. [123], in which the intracranial vault is traced on every tenth slice in the sagittal plane and then their volumes are summed and multiplied by 10 to calculate ICV.

6.1 Analysis:

As previously mentioned, the current anatomical MRI analysis is a part of a larger study on depression. Prior analyses of DTI data and vmPFC volumes are described in Appendix C.

Statistical Analysis

The reliability of the measurements was assessed in two ways on two random selections of 12 images. First, the agreement between total CCA and CC subregion measurements within the same analysis session across the three adjacent slices was assessed. Second, agreement between total CCA and CC subregion areas, averaged from the three slices, was assessed for repeated measures 60 days apart. Both forms of intra-rater reliability

were assessed using intra-class correlations (ICCs) and within-subject coefficients of variation (CVs: standard deviation of within-subject measures as a percentage of their mean).

Clinical and demographic variables were analyzed using independent sample t tests for continuous data, Mann-Whitney U tests for non-parametric data and Chi-square or Fisher's Exact Tests for categorical data. Callosal measurements were generally analyzed using analysis of covariance (ANCOVA) and post hoc least-significant-difference tests. Covariates initially included in the model were ICV, age, gender and years of education. Although ICV was correlated with total CCA, its relationship to CC subregion areas varied and some subregion areas were influenced by age or education in addition to ICV. In contrast, when total CCA was used as a covariate, there were no significant effects of age, education or gender on subregion areas (see Results, section 6.2). Analyses were therefore conducted using total CCA, since this excluded these other influences. Factors included in the analyses were diagnosis (MDD or control), or group (subgroups of MDD, according to type of CIT or medication, and controls). Some additional subgroup comparisons were carried out after examining interactions between sexual/physical abuse (SPA) and emotional abuse/neglect (EAN) in the MDD sample, on the basis that both types of abuse might have more severe effects than either separately.

The statistical threshold was set at two-tailed $p < .05$. A limitation of CC subregion studies is that division into multiple subregions places substantial limitations on study power if a formal Bonferroni correction is employed. For the present study there was reasonable a priori evidence to support the major hypothesis that changes would be present in the rostrum and/or genu and particularly in MDD subjects with CIT. Given the small cell sizes for CIT subgroups, it did not seem appropriate to set a very conservative threshold for

analyses, but any nominally significant results should be considered as more preliminary and exploratory.

Finally, CC genu and rostrum areas were correlated with FA and vmPFC volume measures obtained in the previous analyses, using partial correlations, controlled for ICV, at a statistical threshold of two-tailed $p < .05$, uncorrected.

6.2 Results:

Sample Characteristics

MDD and control subjects were well matched on demographic variables. MDD patients had experienced significantly higher CIT across all types, although controls were not "supranormal" in that a number of controls had also experienced SPA or EAN (Table 6.0). The HDRS scores, frequency of suicide attempts and proportion of subjects with ≥ 3 episodes were consistent with MDD of at least moderate severity.

	Controls	MDD	Statistic	
	n = 34	n = 38		*P*
Age, y	33.5 ± 8.1	33.8 ± 8.0	t = 0.18	.86
Education, y	14.6 ± 1.4	14.4 ± 1.9	t = 0.18	.86
Female, n (%)	27 (79)	29 (76)	$X^2 = 0.01$	.98
Caucasian	29 (85)	32 (84)	$X^2 = 0.01$	.99
Smoker	4 (12)	7 (18)	$X^2 = 0.21$	.65
CTQ total	30 (25 – 35)	53 (39 – 63)	Z = 4.61	< .001
Physical abuse	5 (5 – 7)	6 (5 – 8)	Z = 2.10	.04
Sexual abuse	5 (5 – 5)	6 (5 – 11)	Z = 3.90	< .001
Emotional abuse	5 (5 – 8)	13 (7 – 18)	Z = 4.10	< .001
Emotional neglect	7 (5 – 9)	14 (9 – 18)	Z = 4.17	< .001
Physical neglect	5 (5 – 7)	8 (6 – 10)	Z = 3.86	< .001
SPA, n (%)*	4 (12)	19 (50)	$X^2 = 10.37$	< .001
EAN, n (%)*	5 (15)	21 (55)	$X^2 = 11.10$	< .001
MASQ	104 ± 17	190 ± 31	t = 14.2	< .001
HDRS	NE	20.5 ± 5.4		
Age at onset, y		24.4 ± 9.4		
≥ 3 episodes, n (%)		22 (58)		
Suicide attempts, n (%)†	NE	9 (24)		
Adult trauma, n (%)[I]	NE	11 (29)		
Medicated, n (%)		16 (42)		
Comorbid anxiety, n (%)		25 (66)		
FH of MDD, n (%)	NE	25 (68)		

Table 6.0: Characteristics of Healthy Controls and MDD Subjects

Data are mean ± SD, number (% of group) or median (quartiles)
Abbreviations: MDD = Major Depressive Disorder; CTQ = Childhood Trauma Questionnaire; MASQ = Mood and Anxiety Symptoms Questionnaire; HDRS = Hamilton Depression Rating Scale; FH = family history; NE = Not examined in the current analysis in controls

*Number (%) of subjects in group meeting cut-off for moderate-severe sexual/physical abuse (SPA) or emotional abuse/neglect (EAN).
†Number (%) of subjects in group with at least one lifetime suicide attempt.
[I]Number (%) of subjects in group who had experienced events meeting criterion A of the DSM-IV criteria for PTSD after age 18 years.

MDD Subgroups

MDD subjects with SPA differed on several characteristics from those without, including lower education, smoking, MASQ severity, multiple depressive episodes, suicide attempts and a trend for adult trauma (Table 6.1). Notably, however, they did not differ significantly in the severity of emotional abuse or emotional or physical neglect.

	No SPA	SPA	Statistic	
	n = 20	n = 18		*P*
Age, y	32.5 ± 7.9	35.4 ± 8.0	t = 1.14	.26
Education, y	15.3 ± 1.6	13.9 ± 1.7	t = 2.54	.02
Female, n (%)	14 (70)	15 (83)	Fisher	.45
Caucasian	16 (80)	16 (89)	Fisher	.66
Smoker	0 (0)	7 (39)	Fisher	.003
CTQ total	41 (31 – 53)	59 (47 – 70)	Z = 2.70	.006
Physical abuse	6 (5 – 6)	8 (6 – 13)	Z = 2.86	.004
Sexual abuse	5 (5 – 6)	12 (9 – 17)	Z = 4.93	< .001
Emotional abuse	10 (6 – 19)	16 (10 – 18)	Z = 1.50	.14
Emotional neglect	14 (8 – 17)	14 (11 – 19)	Z = 0.63	.53
Physical neglect	8 (6 – 10)	8 (6 – 10)	Z = 0.28	.80
EAN, n (%)	9 (47)	12 (67)	X^2 = 1.03	.31
MASQ	177 ± 24	203 ± 33	t = 2.74	.01
HDRS	19.7 ± 5.5	21.3 ± 5.5	t = 0.95	.35
Age at onset, y	24.1 ± 8.8	23.4 ± 8.6	t = 0.25	.80
≥ 3 episodes, n (%)	7 (35)	15 (83)	X^2 = 7.20	.007
Suicide attempts, n (%)	2 (10)	8 (44)	Fisher	.03
Adult trauma, n (%)	3 (8)	8 (44)	X^2 = 2.79	.10
Medicated, n (%)	10 (50)	6 (33)	X^2 = 0.50	.48
Comorbid anxiety, n (%)	13 (65)	12 (67)	X^2 = 0.01	.99
FH of MDD, n (%)	14 (74)	11 (61)	X^2 = 0.22	.64

Table 6.1: Characteristics of MDD Subjects with or without History of Childhood Sexual/Physical Abuse (SPA)

Key: See Above

Differences between MDD subjects according to a history of EAN were less marked than those associated with SPA and were limited to higher self-rated symptom severity and trends to earlier onset and more subjects being medicated. Scores on CTQ sexual abuse and physical abuse did not differ significantly.

	No EAN	EAN	Statistic	
	n = 17	n = 21		*P*
Age, y	35.7 ± 7.8	32.3 ± 8.0	t = 1.31	.20
Education, y	14.5 ± 1.8	14.8 ± 1.7	t = 0.59	.56
Female, n (%)	13 (77)	16 (76)	Fisher	.99
Caucasian	15 (88)	17 (81)	Fisher	.67
Smoker	2 (12)	5 (24)	Fisher	.43
CTQ total	38 (30 – 44)	53 (46 – 62)	Z = 4.70	< .001
Physical abuse	6 (5 – 8)	7 (6 – 10)	Z = 1.50	.13
Sexual abuse	5 (5 – 10)	8 (5 – 16)	Z = 1.59	.11
Emotional abuse	7 (6 – 10)	18 (15 – 21)	Z = 4.59	< .001
Emotional neglect	9 (6 – 13)	17 (14 – 21)	Z = 4.41	< .001
Physical neglect	6 (5 – 9)	8 (7 – 11)	Z = 2.61	.009
SPA, n (%)	6 (35)	12 (57)	X^2 = 1.03	.31
MASQ	177 ± 26	199 ± 32	t = 2.34	.03
HDRS	18.9 ± 6.7	21.7 ± 3.9	t = 1.56	.13
Age at onset, y	26.3 ± 7.5	21.7 ± 9.0	t = 1.68	.10
≥ 3 episodes, n (%)	8 (47)	14 (67)	X^2 = 0.79	.32
Suicide attempts, n (%)	2 (12)	7 (33)	Fisher	.15
Adult trauma, n (%)	3 (18)	8 (38)	Fisher	.28
Medicated, n (%)	4 (24)	12 (57)	X^2 = 3.09	.08
Comorbid anxiety, n (%)	10 (59)	15 (71)	X^2 = 0.22	.50
FH of MDD, n (%)	12 (71)	13 (65)	X^2 = 0.01	.99

Table 6.2: Characteristics of MDD Subjects with or without History of Childhood Emotional Abuse/Neglect (EAN)

Key: See Above

Comparison between these subgroups revealed significantly more severe CIT in those who were medicated. Although depressive symptom severity was lower in medicated subjects, morbidity in terms of symptom severity was still notable in view of these subjects having at least 6 months of antidepressant treatment.

	Unmedicated MDD	Medicated MDD	Statistic	
	n = 22	n = 16		*P*
Age, y	35.1 ± 8.0	32.2 ± 7.8	t = 1.09	.28
Education, y	14.5 ± 2.0	14.9 ± 1.5	t = 0.68	.50
Female, n (%)	15 (68)	14 (88)	Fisher	.25
Caucasian	17 (77)	15 (94)	Fisher	.37
Smoker	5 (23)	2 (13)	Fisher	.68
CTQ total	31 (26 – 38)	53 (39 – 63)	Z = 3.79	< .001
Physical abuse	6 (5 – 7)	6 (5 – 8)	Z = 1.22	.22
Sexual abuse	5 (5 – 5)	6 (5 – 11)	Z = 3.29	.001
Emotional abuse	5 (5 – 9)	13 (7 – 18)	Z = 3.34	.001
Emotional neglect	7 (5 – 10)	14 (9 – 18)	Z = 3.31	.001
Physical neglect	5 (5 – 7)	8 (6 – 10)	Z = 3.44	.001
SPA, n (%)*	13 (59)	6 (38)	X^2 = 0.97	.32
EAN, n (%)*	9 (41)	12 (75)	X^2 = 3.09	.08
MASQ	189 ± 25	190 ± 39	t = 0.07	.95
HDRS	22.0 ± 3.7	18.3 ± 6.8	t = 2.15	.04
Age at onset, y	24.8 ± 8.5	22.3 ± 8.8	t = 0.89	.38
≥ 3 episodes, n (%)	14 (64)	8 (50)	X^2 = 0.26	.61
Suicide attempts, n (%)†	6 (27)	3 (19)	Fisher	.71
Adult trauma, n (%)[1]	5 (23)	6 (38)	Fisher	.47
Medicated, n (%)	15 (68)	10 (63)	X^2 = 0.01	.99
Comorbid anxiety, n (%)	13 (62)	12 (75)	X^2 = 0.24	.63

Table 6.3: Characteristics of Unmedicated and Medicated MDD Subjects

Key: See Above

Reliability Data:

Intra-rater reliability measures demonstrate that the current protocol is repeatable with reasonable accuracy by a trained researcher.

Area	Intraclass Correlation	Within subject CV (%)
Total CC	0.98 (0.97 - 0.99)	1.9 (1.7 - 2.2)
Rostrum	0.96 (0.93 - 0.97)	10.4 (9.3 – 11.9)
Genu	0.96 (0.93 - 0.97)	6.0 (5.4 – 6.9)
RB	0.93 (0.89 - 0.96)	5.0 (4.4 – 5.7)
AMB	0.91 (0.86 - 0.95)	5.6 (4.9 - 6.4)
PMB	0.94 (0.90 - 0.96)	5.7 (5.0 – 6.5)
Isthmus	0.78 (0.66 - 0.86)	10.6 (9.4 - 12.3)
Splenium	0.98 (0.97 - 0.99)	2.4 (2.2 – 2.8)

Table 6.4 (a): Intra-Rater Agreement (95% Confidence Limits) for Callosal Volumes on Adjacent Slices

Area	Intraclass Correlation	Within subject CV (%)
Total CC	0.93 (0.77 - 0.98)	2.5 (1.7 - 4.2)
Genu	0.93 (0.77 - 0.98)	6.5 (4.6 - 11.4)
Rostrum	0.92 (0.73 - 0.98)	11.5 (8.0 – 20.3)
G + R	0.97 (0.89 – 0.99)	4.0 (2.8 – 6.9)

Table 6.4 (b): Intra-rater Test-retest Reliability (95% Confidence Limits) for Callosal Volumes

Intracranial Volumes (ICVs)

ANCOVA comparing ICVs between control and MDD subjects showed a significant gender effect (males larger than females) ($F_{1,67} = 35.55$; $p < .001$), but no effects of diagnosis ($F_{1,67} = 0.16$; $p = .69$), age ($F_{1,67} = 0.11$; $p = .75$) or education ($F_{1,67} = 0.35$; $p = .56$).

Diagnosis	ICV (cm^3)
Control	1,385 ± 123
MDD	1,395 ± 126

Table 6.5: Comparison of ICVs in Control and MDD Groups

Similarly, comparisons of control and MDD subjects with or without SPA showed a gender effect (M>F) ($F_{1,66} = 34.79$; $p < .001$), but no effects of group ($F_{2,66} = 0.10$; $p = .91$), age ($F_{1,66} = 0.10$; $p = .75$) or education ($F_{1,66} = 0.37$; $p = .54$).

Group	ICV (cm^3)
Control	1,385 ± 123
MDD without SPA	1,392 ± 121
MDD with SPA	1,398 ± 135

Table 6.6: Comparisons of ICVs in Controls, MDD with and without SPA

Comparison of control and MDD subjects with or without emotional abuse/neglect also showed a gender effect (M>F) ($F_{1,66} = 34.93$; $p < .001$), but no effects of group ($F_{2,66} = 0.08$; $p = .92$), age ($F_{1,66} = 0.10$; $p = .75$) or education ($F_{1,66} = 0.34$; $p = .56$).

Group	ICV (cm^3)
Control	1,385 ± 123
MDD without EAN	1,395 ± 121
MDD with EAN	1,395 ± 134

Table 6.7: Comparisons of ICVs in Controls, MDD with and without EAN

In summary, although males (1,529 ± 102 cm^3) had larger ICVs than females (1,351 ± 100 cm^3), there were no significant group differences in ICV.

Covariates of CC Volumes

Within the whole sample, total CC volume was significantly predicted by ICV (positive), age (negative) and education (negative), but not gender.

Covariate	df	F	P
ICV	1	8.32	***.005***
Age	1	8.45	***.005***
Gender	1	0.92	.34
Education	1	4.98	***.03***

Table 6.8: Significance of Covariates with Total CC Volume

Multivariate ANCOVA for CC subdivisions, including covariates of ICV, age, sex and education, indicated that ICV and age tended to exert effects across multiple CC subregions.

Covariate	df	F	P
ICV	7, 61	2.28	***.04***
Age	7, 61	2.09	***.06***
Gender	7, 61	1.64	.14
Education	7, 61	1.31	.26

Table 6.9: Multiple Analysis of Covariance for CC Subregions

However, follow up univariate ANCOVAs for CC subdivisions indicated that although ICV, age and education, but not gender, were associated with variations in individual CC subdivision volumes, these effects varied between subdivisions and specific covariates.

Covariate	Subdivision	df	F	P
ICV	Genu	1,67	10.09	.002
	Isthmus	1,67	3.47	.07
	Splenium	1,67	9.29	.003
Age	Genu	1,67	2.93	.09
	Rostral Body	1,67	10.10	.002
	Anterior Midbody	1,67	11.55	.001
	Posterior Midbody	1,67	3.37	.07
	Isthmus	1,67	3.89	.05
	Splenium	1,67	3.92	.05
Education	Rostrum	1,67	4.05	.05
	Anterior Midbody	1,67	5.80	.02

Table 6.10: Analysis of Covariates in CC Subregions- Significant Associations

In contrast, when CC subdivisions were analyzed using MANCOVA with total CC volume as a covariate, in addition to covariates of age, sex and education, total CC volume was a significant covariate for multiple subdivisions ($F_{7,61} = 2{,}215$; $p < .001$), whereas age ($F_{7,61} = 0.97$; $p = .46$), gender ($F_{7,61} = 1.50$; $p = .18$) and education ($F_{7,61} = 0.97$; $p = .46$) were not. Follow-up univariate ANCOVAs indicated that all CC subdivision volumes were

related to total CC volume, whereas for other covariates, there was only a trend relationship between age and anterior midbody volume.

Covariate	Subdivision	df	F	P
Total CC volume	Rostrum	1,67	10.74	.002
	Genu	1,67	139.95	< .001
	Rostral Body	1,67	52.92	< .001
	Anterior Midbody	1,67	100.91	< .001
	Posterior Midbody	1,67	115.21	< .001
	Isthmus	1,67	65.59	< .001
	Splenium	1,67	121.62	< .001
Age	Anterior Mid-Body	1,67	3.26	.08

Table 6.11: Relationship of Callosal Subdivision Volumes to Total CC Volumes and Age

In summary, although total CC volume was related to brain size, as measured by ICV, this relationship was not consistent across all CC subdivisions. Although there were no gender differences in total CC volume after covarying for ICV, total CC volume was inversely related to age and years of education. ICV, age and education did not show consistent relationships across all CC subdivisions. Covarying for total CC volume, rather than ICV, removed any influences of age, gender or education on the volumes of CC subdivisions.

Comparison of Total CC Volumes between Groups

Total CC volumes did not differ between controls and MDD subjects, including subgroups of MDD subjects according to CIT history or medication (see Table 6.12).

Group	n	Total CC volume (mm^3)	df	F	p
Control	34	311.0 ± 46.3	1,67	0.82	.37
MDD	38	302.4 ± 42.7			
Control	34	311.0 ± 46.3	2,66	0.89	.42
MDD, no SPA	20	309.1 ± 36.1			
MDD with SPA	18	295.8 ± 48.3			
Control	34	311.0 ± 46.3	2,66	0.56	.57
MDD, no EAN	17	298.3 ± 41.0			
MDD with EAN	21	305.7 ± 44.0			
Control	34	311.0 ± 46.3	2,66	0.51	.60
MDD, unmedicated	22	305.0 ± 43.9			
MDD, medicated	16	311.0 ± 46.3			

Table 6.12: ANCOVA of Total CC Volumes, Including ICV, Age and Education as Covariates in the Model.

Comparison of CC Subdivision Volumes between Groups

There were no significant differences in CC subdivision volumes between MDD subjects and controls.

CC subdivision volumes (mm^3)	Control n = 34	MDD n = 38	df	F	P
Rostrum	12.4 ± 5.4	13.6 ± 5.5	1,69	1.02	.32
Genu	61.6 ± 13.9	63.4 ± 13.6	1,69	0.98	.33
Rostral Body	47.1 ± 8.7	47.2 ± 7.5	1,69	0.01	.93
Anterior Midbody	36.5 ± 5.9	36.8 ± 6.7	1,69	0.16	.69
Posterior Midbody	33.1 ± 6.2	33.5 ± 6.3	1,69	0.19	.66
Isthmus	26.9 ± 6.5	25.8 ± 4.7	1,69	1.53	.22
Splenium	88.9 ± 14.4	86.6 ± 10.8	1,69	1.70	.20

Table 6.13: Comparison of CC Subdivision Volumes between Controls and MDD Subjects, including Total CC Volume as a Covariate in the Model.

Although the overall F ratio test did not quite reach the threshold for significance, MDD with SPA subjects had higher covariate-adjusted rostrum volumes than MDD without SPA or controls (Table 6.14). Given this difference in rostral volume relative to total CC

volume, rostral volumes adjusted for ICV and absolute rostral volumes were also examined. ICV-adjusted rostrum volumes were also larger in MDD with SPA (15.0 ± 6.4 mm^3), compared with MDD without SPA (11.9 ± 4.3 mm^3) or controls (12.7 ± 5.4 mm^3), but these differences did not reach significance (p = .07 and .14 respectively). Absolute values for rostrum volume were also larger in MDD with SPA (15.0 ± 6.4 mm^3), compared with MDD without SPA (12.0 ± 4.3 mm^3) or controls (12.6 ± 5.4 mm^3), but these differences were not significant (p = .09 and .14 respectively).

CC subdivision volumes (mm^3)	Control n = 34	MDD, no SPA n = 20	MDD with SPA n = 18	df	F	p
Rostrum	12.4 ± 5.4	12.0 ± 4.3	*15.5 ± 6.4**	2,68	2.93	.06
Genu	61.6 ± 13.9	63.7 ± 12.5	63.0 ± 15.3	2,68	0.53	.59
Rostral Body	47.0 ± 8.7	46.9 ± 7.3	47.5 ± 7.7	2,68	0.06	.94
Anterior Mid-Body	36.5 ± 5.9	36.9 ± 6.0	36.8 ± 7.5	2,68	0.08	.92
Posterior Mid-body	33.1 ± 6.2	33.8 ± 5.6	33.1 ± 7.0	2,68	0.23	.80
Isthmus	26.9 ± 6.5	25.5 ± 3.6	26.1 ± 5.8	2,68	0.84	.44
Splenium	88.9 ± 14.4	87.6 ± 10.8	85.6 ± 10.6	2,68	1.19	.31

Table 6.14: Comparison of CC Subdivision Volumes between Controls and MDD Subjects, with or without History of Childhood Sexual/Physical Abuse (SPA), including Total CC Volume as a Covariate in the Model.

*MDD with SPA > controls, p = .04; MDD with SPA > MDD, no SPA, p = .03.

There were no significant differences in CC subdivision volumes between controls and MDD subjects with or without history of childhood EAN, although there was a trend for MDD subjects with a history of childhood EAN to have a smaller splenium volume than controls (see *, Table 6.14). Absolute splenial volumes did not differ between MDD subjects with EAN (85.7 ± 10.8 mm^3), compared with MDD without EAN (85.9 ± 11.2 mm^3; p = .96) or controls (89.9 ± 14.4 mm^3; p = .24).

CC subdivision volumes (mm^3)	Control n = 34	MDD, no EAN n = 17	MDD with EAN n = 21	df	F	P
Rostrum	12.4 ± 5.4	13.1 ± 5.0	14.0 ± 6.0	2,68	0.65	.52
Genu	61.6 ± 13.9	63.6 ± 12.9	63.2 ± 14.3	2,68	0.50	.61
Rostral Body	47.1 ± 8.7	46.5 ± 8.3	47.7 ± 6.7	2,68	0.19	.83
Anterior Mid-body	36.5 ± 5.9	36.7 ± 6.4	36.9 ± 7.0	2,68	0.09	.91
Posterior Midbody	33.1 ± 6.2	33.0 ± 6.6	33.8 ± 6.0	2,68	0.33	.72
Isthmus	26.9 ± 6.5	25.9 ± 3.9	25.7 ± 5.3	2,68	0.77	.47
Splenium	88.9 ± 14.4	88.6 ± 11.2	*85.1 ± 10.8**	2,68	1.92	.16

Table 6.15: Comparison of CC Subdivision Volumes Between Controls and MDD Subjects, with or without History of Childhood Emotional Abuse/Neglect (EAN), including Total CC Volume as Covariate in the Model.

*MDD with EAN < controls, p = .07.

Medicated MDD subjects had a larger anterior mid-body volume than controls and unmedicated MDD subjects, who did not differ.

CC subdivision volumes (mm^3)	Control n = 34	MDD, unmedicated n = 22	MDD, medicated n = 16	df	F	P
Rostrum	12.4 ± 5.4	14.7 ± 6.4	12.2 ± 3.7	2,68	1.67	.20
Genu	61.6 ± 13.9	64.6 ± 13.5	61.6 ± 13.8	2,68	1.16	.32
Rostral Body	47.1 ± 8.7	47.0 ± 8.0	47.5 ± 7.1	2,68	0.04	.97
Anterior Mid-Body	36.5 ± 5.9	35.5 ± 6.3	*38.7 ± 7.2**	2,68	3.53	.04
Posterior Midbody	33.1 ± 6.2	33.0 ± 7.1	33.1 ± 5.3	2,68	0.32	.73
Isthmus	26.9 ± 6.5	25.8 ± 4.6	25.8 ± 5.0	2,68	0.76	.47
Splenium	88.9 ± 14.4	88.6 ± 11.2	85.1 ± 10.8	2,68	0.84	.44

Table 6.16: Comparison of CC Subdivision Volumes between Controls and Unmedicated or Medicated MDD Subjects, including Total CC volume as a Covariate in the Model.

*medicated MDD > controls, p = .05; medicated MDD > unmedicated MDD, p = 0.01.

In summary, there were no differences in total CC volume associated with MDD or childhood maltreatment and there were no significant differences in CC subdivision volumes

associated simply with a diagnosis of MDD. However, rostrum volumes tended to be greater in MDD subjects with a history of childhood SPA and splenium volumes tended to be smaller in MDD subjects with a history of EAN. Antidepressant treatment was associated with larger anterior midbody volumes.

Given the evidence for larger rostrum volumes and a trend for smaller splenium volumes associated with CIT, these regions were examined in more detail. Rostrum volumes in MDD subjects were analyzed using ANCOVA to test the main effects and interactions of SPA and EAN, including total CC volume as a covariate. This analysis showed a main effect of SPA ($F_{1,33} = 4.40$; $p = 0.04$), but no main effect of EAN ($F_{1,33} = 0.01$; $p = 0.97$) or SPA x EAN interaction ($F_{1,33} = 0.14$; $p = 0.71$). Rostrum volumes were larger in MDD subjects with SPA both with or without EAN than in MDD subjects without SPA.

Factors		n	Rostrum Volume (mm^3)
No SPA	No EAN	11	12.0 ± 4.8
	With EAN	9	11.4 ± 3.9
With SPA	No EAN	6	14.9 ± 5.7
	With EAN	12	15.5 ± 6.7

Table 6.17: Rostrum Volumes in MDD Subjects According to History of Child Maltreatment, Adjusted for Covariate of Total CC volume.

Splenium volumes in MDD subjects were also analyzed using ANCOVA to test the main effects and interactions of childhood sexual/physical abuse (SPA) and emotional abuse/neglect (EAN), including total CC volume as a covariate. The main effects of SPA ($F_{1,33} = 0.17$; $p = 0.69$) and EAN ($F_{1,33} = 2.73$; $p = 0.11$) were not significant, but there was an SPA x EAN interaction ($F_{1,33} = 3.98$; $p = 0.05$). Only MDD subjects with histories of both SPA and EAN had small splenium volumes.

Factors		n	Splenium Volume (mm^3)
No SPA	No EAN	11	86.4 ± 11.4
	With EAN	9	87.2 ± 10.7
With SPA	No EAN	6	89.8 ± 10.5
	With EAN	12	82.1 ± 11.1

Table 6.18: Splenium Volumes in MDD Subjects According to History of Child Maltreatment, Adjusted for Covariate of Total CC volume

These data suggested that only MDD with both SPA and EAN combined might be associated with reduced splenium volumes. When the data from the whole sample were re-analyzed to compare controls, MDD subjects without combined maltreatment and MDD subjects with combined maltreatment, there was a significant group effect ($F_{2,68} = 3.30$; $p = 0.04$), with MDD subjects with combined maltreatment showing lower splenium volumes than controls ($p = .02$) and the other MDD subjects ($p = .03$). However, it should be noted that splenium volumes in subjects with combined maltreatment (84.3 ± 11.1 mm^3) were not significantly reduced when ICV was included as the covariate in the model, either compared with controls (90.2 ± 14.4 mm^3; $p = .15$), or the other MDD subjects (86.1 ± 10.8 mm^3; $p = .67$). Nor were absolute splenial volumes (84.2 ± 11.1 mm^3) significantly reduced, compared with controls (89.9 ± 14.4 mm^3; $p = .18$), or the other MDD subjects (86.5 ± 10.8 mm^3; $p = .67$).

Correlation Analysis

Using the data obtained in the prior DTI and vmPFC volume studies (Appendix C), partial correlations between rostrum and genu (together), and rostrum CC volumes, FA of medial prefrontal and ventromedial prefrontal WM were examined, controlling for total CC volume. Correlations between rostrum volume and ventromedial prefrontal cortex (vmPFC)

grey matter volume were also examined, controlling for ICV, which influences both. These analyses were conducted in the whole sample and in the controls and MDD subjects separately. Volume of the rostrum was found to be inversely correlated with ventromedial prefrontal FA in the whole sample and in the MDD subjects, but not in healthy controls (see Table 6.19). These data suggest that the increase in rostrum volume and decrease in ventromedial prefrontal FA in the group of MDD subjects with histories of SPA may be associated. As EAN was not shown to have an effect on rostrum values, correlations were not calculated. vmPFC GM volumes were not found to be correlated with rostrum volumes.

Group	Region	Correlation Area	R	p
Whole sample	Rostrum	vm prefrontal FA	-.28	.04
	Genu + Rostrum	Medial prefrontal FA	-.10	.48
	Rostrum	vmPFC GM volume	.04	.72
Controls	Rostrum	vm prefrontal FA	-.15	.46
	Genu + Rostrum	Medial prefrontal FA	-.27	.18
	Rostrum	vmPFC GM volume	.18	.33
MDD	Rostrum	vm prefrontal FA	-.35	.07
	Genu + Rostrum	Medial prefrontal FA	-.27	.18
	Rostrum	vmPFC GM volume	-.03	.86

Table 6.19: Partial Correlations of Rostrum and Genu + Rostrum Volumes with FA of Medial and Ventromedial Prefrontal FA (vm Prefrontal FA) (Controlling for Total CC Volume) and vmPFC GM Volume (Controlling for ICV)

Chapter 7

Discussion

7.0 Interpreting the Findings

This study evaluated the differences in the volume of the midsagittal CC and the relative volume of its subregions between patients diagnosed with MDD, including subgroups with and without histories of CIT, and matched control subjects.

As expected, a gender difference was found in ICV, as can be explained by allometry (men have larger bodies than women). However, neither analysis of MDD versus controls, nor subgroups of MDD subjects with SPA or EAN versus controls yielded significant differences for ICV. This indicates that development of total brain volume, for which ICV is a proxy, was not associated with diagnosis or a history of childhood CIT.

Examinations of total CC volume indicated that it was predicted positively by the covariate of ICV, negatively by age and education, but not by gender. Similar examinations of CC subregions suggested that ICV, age and education were associated with variations in the individual subregions, and also indicated that gender was not a determining factor. The positive associations with ICV are expected as a simple correlate of brain size. The negative correlation with age is consistent with findings in a recent study of a large ($n = 432$) normative general population sample [48]. Although education was not significantly associated with total CCA in the general population study, in the present sample age and years of education were correlated ($r = 0.44$; $p < .001$), which may perhaps reflect a selection bias due to recruitment of younger and more educated subjects from university and hospital

settings. Smoking was not associated with significant differences in total CC volume in the present sample or with CCA in the large normative sample.

Covariate-adjusted total CC volumes did not significantly differ between controls and MDD subjects, which is in agreement with previous studies (see Table 4.0). Total CC volumes also did not differ between unmedicated and medicated MDD subjects. Finally, total CC volume was not significantly different in the EAN and SPA subgroups of MDD subjects. These findings are consistent with prior studies of MDD, which did not report any global changes in CCA. Although pediatric studies of CIT have reported reduced CCA, the findings have been less clear in adults with a history of CIT, with one study reporting reduced CCA [111] in a sample of whom 50% had a history of CIT, and one study reporting no change in CCA [112]. However, both studies included only small samples (see Table 4.1). The pediatric studies included subjects with severe maltreatment, brought to the attention of authorities and resulting in social services intervention and/or admission for psychiatric treatment. Reductions in total CCA might therefore be limited to subjects who experienced more severe abuse or neglect, or might be related to age at abuse, since the mean ages in these studies were 12-13 years. It is possible that abuse and neglect in later childhood may be associated with less effect on CCA and a limitation of using the CTQ to assess CIT is that it does not assess the ages at which abuse and neglect occurred.

No significant differences in callosal subregions were found between controls and MDD subjects as a single group. However, the major hypothesis of the study was that MDD is heterogeneous and that changes in anterior CC volume may be limited to MDD subjects with a history of CIT. The findings were consistent with this hypothesis, in that the MDD subgroup with SPA had significantly larger rostrum volumes than either healthy controls or MDD subjects without SPA, when covariates were taken into account. Although

ICV-adjusted and absolute rostrum volumes were also larger in MDD subjects with SPA, the finding was more robust statistically when total CC volume was used as the covariate. A history of EAN was not found to be associated with differences in rostrum volume, either alone or in combination with SPA, and differences in the volume of the genu and rostral body were not found. The latter findings did not seem to be a consequence of limited power, since the differences in volumes were very small.

MDD subjects with histories of EAN showed a trend towards reduced splenial volumes compared with controls. However, this difference was significant only when total CC volume was included as a covariate in the model, with no significant differences in ICV-adjusted or absolute splenium volumes. Furthermore, EAN alone did not clearly differentiate a subgroup of MDD subjects with reduced splenium volume, since volumes were not significantly different from those in MDD subjects without EAN. However, there was an interaction between SPA and EAN, with MDD subjects who experienced both having the lowest splenium volumes. It was less clear than for the rostrum that these subjects formed a robust subgroup of MDD patients, since they only separated from other MDD subjects when total CC volume was included as a covariate.

The other finding that may be noted in the present study is that there were no differences in the volume of posterior midbody in MDD associated with CIT, although this region has been implicated in previous pediatric and adult studies. This might be related to differences in CIT between studies in terms of developmental age, severity or type. For example, Teicher et al. [27] reported that severe physical neglect had the greatest impact in their pediatric sample, but physical neglect was of low severity in the current sample. A further factor is that MDD subjects with severe personality disturbance were not included in

the present study. Reduced parietal lobe volumes have been reported in borderline personality disorder [124], which is commonly associated with histories of severe CIT.

Antidepressant treatment was associated with larger anterior midbody values. Although this is a novel finding, it should be regarded cautiously, since no specific medication effects were hypothesized. Additionally, there were multiple antidepressant regimens used in the study population. As the anterior midbody is the callosal region primarily involved in connecting the motor cortices, it might be speculated whether changes are associated with antidepressant effects on psychomotor retardation or agitation. However, medicated patients also had a larger anterior midbody on average than controls, who did not differ from unmedicated patients, arguing against the idea that medication was reversing or preventing an abnormality in MDD. Since multiple exploratory analyses were conducted, this may have been a chance finding.

MDD subjects with SPA showed both increased volume of the rostrum and lower FA of white matter tracts that project through the rostrum and inferior portion of the genu to ventral and medial portions of the prefrontal cortex. Furthermore, these measures were inversely correlated in MDD, more weakly correlated in the whole sample and uncorrelated in controls. This suggests that the association may have be caused by the relatively low FA and high rostrum volumes seen in the MDD subjects with a history of SPA. The FA of medial prefrontal WM, which is calculated from all WM traversing the genu and rostrum, was not related to the total volume of genu and rostrum. This suggests that more dorsal WM that crosses the upper portion of the genu might be less affected. A caveat is that FA was measured throughout the portion of the tract that is proximal to the CC. It is possible that FA and subregion volume might have shown different relationships if FA had been measured from voxels within genu and rostrum regions of interest on a midsagittal slice.

However, this additional analysis was not possible within the time available. The current DTI data are less suitable for volume measures, due to their lower spatial resolution (2.0 x 2.0 x 2.0 mm^3) and because definition of the MSS may be less precise than for MRI, as landmarks are less clearly visualized on DTI images (compare Figures 6.1, C.0).

MDD patients showed reduced vmPFC GM volume associated with combined SPA and EAN, but vmPFC GM volume and rostrum volumes were not correlated. Unfortunately vmPFC WM volumes were not traced, although these might have a more direct relationship. Furthermore, although the rostrum contains a portion of the commissural WM that connects left and right ventromedial PFC, it is only a portion of this WM, which also traverses the ventral genu. This limited correspondence between measures reflect a fundamental limitation of MRI measures of CC subregions, which is that it requires the use of landmarks or proportions that are semi-arbitrary. Although they bear topographical relationships to cortical regions on average [51] the correspondence is not precise.

The differences in rostrum volume and ventromedial prefrontal FA associated with MDD and SPA suggest the possibility that information transfer between the homologous caudal/orbital prefrontal cortices would be affected. Although the composition of the callosal subregions has been characterized, the functional significance of the effects of volume differences and small differences in FA are still unclear [24]. However, recent DTI microstructural research has linked WM integrity of the CC to neuropsychological performance in adolescents. Fryer et al. [16] found that splenium integrity was associated with language and psychomotor function, while visuospatial construction abilities were associated with callosal midbody and splenium integrity (n =18).

The data are also consistent with neuroanatomical, neuropsychological and functional neuroimaging evidence that prefrontal abnormalities are common in MDD [82]. The prefrontal cortex plays important roles in the evaluation of social and emotional stimuli and in the regulation of emotional responses. PET studies have shown that depressed patients have abnormal PFC glucose metabolism, specifically in ventromedial and anterior cingulate cortex, orbitofrontal cortex and lateral cortex [125]. Each specific region of the PFC has its own specialized function related to emotional regulation. The lateral prefrontal cortex seems to be involved in attention and executive control, the orbitofrontal cortex in the determination of relative reward and punishment values, needed for selection of goals and responses, whereas the ventromedial and anterior cingulate cortex may play a more direct role in the generation and regulation of emotional responses. There is evidence that the left PFC and right PFC have different functions, with the left PFC more involved in generating positive emotions and approach behaviors, while the right PFC has been associated with negative emotions and avoidance, mainly based on EEG studies [126]. It might be speculated that altered interhemispheric connections could contribute to impaired integration and imbalance between these tendencies, contributing to symptoms of MDD. The ventromedial PFC also has connections with the hypothalamus and may play a role in the HPA axis dysregulation seen in MDD, which appears to be more marked in subjects with a history of CIT [127].

7.1 Effects of SPA and EAN

Social experiences throughout life can influence genetics and behaviour but it has been shown that interactions at a young age are particularly important. This highly plastic period is important in determining neuronal differentiation, maturation and synaptic

connections [128]. Children who experience severe disturbances in early care have been shown to be at a higher risk of behavioural and social problems, as well as of psychiatric diseases [129].

While reports of SPA prevalence vary, it has been estimated that in women there is an 8%-32% likelihood while in men the frequency is about 1-16% [106]. In the current investigation, SPA was associated with a difference observed in the rostrum of the corpus callosum in the patient groups. This finding of increased rostrum volume is in accordance with some reports finding anterior CC changes in MDD populations [24, 95]. However, in previous reports regarding CIT, it has been associated with callosal size decreases and not specifically in the rostrum (see Table 4.1) [27,109]. It is possible that in part this reflects developmental age, since it has been found that there is a posterior to anterior gradient in WM development [29]. PFC white matter develops slowest, with maturation continuing into adulthood. Studies in pediatric samples would not capture the impact of CIT on later maturing regions of WM, although the data from De Bellis et al. [109] suggested an age x diagnosis interaction affecting rostral area. Differences between samples in primary diagnosis, CIT severity and developmental age during occurrence of CIT may also be important. Furthermore, not all of the impact of CIT will be direct, since it may be a marker for exposure to subsequent adversity. For example, MDD subjects in the present study who were exposed to childhood SPA also more frequently experienced adult traumatic stressors.

Although the effect of early emotional and physical neglect on neurobiological sequelae has been less studied than that of abuse, as an early adverse experience it may be more prevalent [27]. Neglect differs from abuse due to its nature; it is associated with a lack of stimulation and interaction. The current analysis did not clearly identify specific associations with EAN, since differences in splenium volume were associated more with

combined SPA and EAN than with EAN alone. However, it could be speculated that neglect might be more associated with posterior CC development because a lack of interaction and sensory stimulation impairs development of sensory cortices.

It is difficult to obtain data on human interactions in a controlled environment, but animal studies may be relevant. For example, in male Rhesus monkeys, smaller CC were found in those monkeys raised individually under laboratory conditions vs. those raised in semi-natural environments, and these differences persisted even after reintroduction to peer-housing [130]. In rodents, studies where the mother-infant interaction has been manipulated have resulted in persistent behavioural and neuroendocrine responses throughout the lifetime [131, 132]. Studies by Branchi [133, 134] have indicated the importance of early social enrichment (via maternal and peer interactions) with regard to neurobiological development, showing that mice raised in highly stimulating early social environments were more likely to socially interact and more efficiently able to establish their social roles. Also found to be increased were brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) levels. As impairments in social behavior and altered neurotrophin levels (particularly BDNF) have been linked with psychiatric diseases, including depression and schizophrenia [135, 136, 137, 138] it follows that the early social environment plays a role in the susceptibility of individuals to develop psychopathology [134].

Specifically to the CC, preclinical studies have shown that CC size was impacted by early experience with a gender-dependent effect [139, 140]. Enriched environments resulted in larger, more regular CC in male, but not female rats [139]. Additionally, rats of both sexes reared in enriched environments had larger and greater numbers of unmyelinated axons than those reared in isolated environments. It was also suggested that in females, early environments affect the number of myelinated axons where in males the diameter of

myelinated axons are affected. However, the relationship of these animal models to human development and clinical disorders may be tentative.

While it was not the aim of the present study to examine differences in gender, which was not a significant covariate of CC volumes, it is well established that the prevalence of depression is greater in women [141]. While the reasons for this have not been completely elucidated there are several possible components that contribute to this difference. It is thought that the occurrence of stressful life events, hormonal influences or sexual dimorphisms in the brain are involved [90]. In their 2004 study, Teicher et al. [27] found that neglect had the greatest influence on the CC in boys, with girls being more affected by SPA. Similarly, De Bellis and colleagues [108] found the CC to be more affected by neglect in male subjects than females. However, given the small cell sizes of subgroups already examined in the present study and the small number of males, it was not possible to examine any interactions with gender. Still, two males in the present sample met the criteria for sexual abuse, so this factor was not unique to female participants.

7.2 Implications of the DTI, GM and MRI Studies Combined

Although this study and the literature indicate that there is a relationship between childhood maltreatment and size of the CC, there is no proof of a direct cause-effect relationship, since maltreatment is often associated with other current and later stressors and with subsequent alterations in stress perception and response, for example, within the HPA axis [27].

An important finding in the present study was that the volume of the rostrum was associated with the FA of ventromedial prefrontal WM and that this difference was driven by a correlation in MDD patients, presumably related to the more extreme values in MDD

subjects with SPA. This result might reflect on the organization and makeup of axonal fibres in the CC and surrounding WM. In prefrontal regions, the proportion of thin, slow conducting axons is known to be higher relative to larger myelinated axons and FA in the anterior CC is lower than more posteriorly, but increases with development [7]. There is some evidence that axonal structure has a larger influence on FA than myelination [142], so that reduced FA associated with SPA may also be associated with a higher proportion of thinner, more loosely packed unmyelinated axons, consistent with the idea of delayed maturation. An increase in rostrum volume in association with SPA could be due to several possibilities-- less densely organized axonal packing, more axons, or a higher proportion of larger myelinated axons. The last possibility seems the least consistent with the FA data, suggesting that there might be more axons (perhaps associated with delayed or impaired pruning) [116], and/or a higher proportion of more loosely packed axons. Other possible considerations are abnormally decreased amounts of axonal myelination or pruning. These changes may reflect an impact of adversity on the maturation processes of WM. Closer integration between the different imaging methods in future studies may help to clarify how microstructural and volumetric measures are related. Although there was no relationship detected between rostrum volume and vmPFC volume in the present study, this was addressed only in a very preliminary fashion since vmPFC white matter volume was not analyzed. An increased rostrum volume and decreased vmPFC volume were both present at the group level, but this did not play out at the level of individual differences, perhaps due to the reasons discussed above.

Chapter 8

Conclusion

8.0 Summary

Taken together, the literature and the present data point to the idea that the maturation processes of the CC are somehow disrupted by abuse and neglect. Human and animal studies indicate that early adverse experiences can have significant effects on future neurodevelopment. Researchers have begun to explore childhood maltreatment as a factor in alterations in white and gray matter morphology in psychiatric disorders, but as yet there are few studies in the field and the sample sizes of these studies tend to be small (including the present study). There are limitations to the interpretation of cross-sectional data. Large prospective follow up studies of pediatric samples are needed to clarify how early adversity may impact development, although such studies would be very challenging to conduct.

The results suggest that patients with childhood experiences of SPA or EAN show callosal and other neurobiological changes, but are not clear in determining their relationships to MDD. Depression is not necessarily a direct consequence of childhood maltreatment; and complex interactions of multiple factors are likely to play contributing roles. Moreover, the present results indicate that size differences in the CC may not be a direct consequence of psychiatric illness and changes seen in depressed subjects may be due to underlying factors such as CIT rather than psychiatric illness itself. Including a control group of CIT-exposed subjects without MDD or major psychiatric disorders would help to clarify whether callosal changes and mental health impact are related. Childhood adversity is also a risk factor for PTSD and other psychiatric disorders, indicating that the specificity of

associations needs to be further examined. It is possible that changes observed in the CC or other neurobiological variables in MDD involve interactions between genetic and experiential factors. However, increasing the number of factors to be examined within a neuroimaging study presents great challenges in terms of sample size.

Nevertheless, these findings highlight the importance of coming to an understanding of CIT related changes and studying pediatric populations with these implications in mind. Although the current study focuses on WM changes in the CC, it is important to remember that CIT may have far-reaching consequences on human behaviour. Aside from mental problems, victims of CIT are faced with issues such as physical health problems, substance abuse, victimization, and criminality in adulthood [143, 144].

It is the hope that this research will ultimately provide scientific knowledge which can then be used by policy makers to increase the inclusion of CIT education into curriculums in not only the medical and mental health fields, but public education [103]. This awareness will hopefully result in the introduction of intervention and prevention efforts and lead to a better prognosis for victims and patients in the future.

8.1 Contribution

This study links neurobiological differences between MDD patient and normative groups with childhood maltreatment. In particular, it suggests that MDD is heterogeneous and implicates sexual and physical abuse, and less clearly emotional abuse and neglect to changes in callosal morphology. The localization of changes to the rostrum of the CC is consistent with other evidence for changes in ventromedial and orbitofrontal structure and function in MDD, suggesting that the role of CIT in these other changes should be

examined more closely. Whilst the direction of changes in anterior CC in MDD has been inconsistent, there are few studies to date, with wide variations in sample composition.

To the author's knowledge, this is the first study of its type examining a MDD group and linking changes in the CC to histories of childhood maltreatment. In addition, the findings in this study indicate that a history of CIT may be one of the reasons for disparate results seen in the literature thus far. It has shown that the division of the MDD subgroup into those who have experienced childhood maltreatment and those who have not can reveal differences in callosal volume and microstructure that would not otherwise be evident.

A strength of the current study is that both anatomical MRI and DTI approaches were used to study WM and GM, assisting the interpretation of the findings as suggesting delayed maturation.

8.2 Limitations and Future Directions

There are a number of limitations that must be considered with regards to the current study. Firstly, the sex distribution of the sample was imbalanced and the numbers of males too small to examine sex differences. As sexual abuse is more likely to occur in females, additional difficulties would be presented in trying to compare specific types of abuse across sexes and to match for severity. Even though the subtyping of CIT abuse was simplified into two groups, another limitation of the sample was that these subgroups were small in size, particularly to examine interactions between SPA and EAN. Although the size of the overall sample was comparable to previously published work, much larger samples would be needed to achieve substantial statistical power, particularly to control for multiple comparisons. Power would be further challenged by including additional factors, notwithstanding current research interest in gene x environment interactions in MDD.

This investigation was also limited in terms of the retrospective assessment of CIT. Although the CTQ has good psychometric properties and is considered valid, it does not assess the developmental age at which CIT occurred. This is reasonable in the sense that adult subjects may more accurately recall that CIT was an infrequent or common experience than specific details of when and where.

This study also made use of manual segmentation techniques to find volumes of callosal areas. Although every effort was made to control for error and standardize procedures and intra-rater reliability was good, the possibility of human error remains. An automated segmentation protocol should be more reliable, but validity may still require manual checking.

A number of avenues for future research are suggested by the present findings. Aside from increasing power by recruiting larger samples, research focusing on the areas implicated here warrants attention. As advances in DTI technology will hopefully allow the characterization of white matter changes seen in the current investigation, functional testing of the cortical areas implicated will be able to relate changes in structure to changes in behaviour. This functional testing may be possible by combining DTI and fMRI technologies. Neuropsychological testing of subjects may also reveal whether differences in anterior callosal volumes are associated with differences in performance. Although the prefrontal cortex has been frequently studied in MDD and is beginning to be examined in CIT, the prior literature on CIT also suggests changes in posterior regions of CC, arguing that parietal, temporal and occipital cortex differences may be relevant to this population.

Finally, while further scientific studies will be conducted, quantitative values alone may not be able to provide answers to all of the questions. It is likely that studies combining qualitative and quantitative approaches will be necessary to further this research. Increased

information about particular experiences of CIT could help identify windows of vulnerability to changes in morphology. This increased detail combined with advances in technology and methods may contribute to significant progress in this field.

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Appendix A

A 1 Diagnostic Criteria of Major Depressive Disorder

(Adapted from DSM-IV [78])

Major Depressive Episode

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.

Note: Do not include symptoms that are clearly due to a general medical condition, or mood-incongruent delusions or hallucinations.

(1) Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful).

Note: In children and adolescents, can be irritable mood.

(2) Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)

(3) Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.

Note: In children, consider failure to make expected weight gains.

(4) Insomnia or hypersomnia nearly every day

(5) Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)

(6) Fatigue or loss of energy nearly every day

(7) Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)

(8) Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)

(9) Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

B. The symptoms do not meet criteria for a Mixed Episode.

C. The symptoms cause clinically significant distress or impairment in social,

occupational, or other important areas of functioning.

D. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).

E. The symptoms are not better accounted for by Bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.

Major Depressive Disorder

Single Episode

A. Presence of a single Major Depressive Episode

B. The Major Depressive Episode is not better accounted for by Schizoaffective Disorder and is not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Psychotic Disorder Not Otherwise Specified.

C. There has never been a Manic Episode, a Mixed Episode, or a Hypomanic Episode.

Note: This exclusion does not apply if all the manic-like, mixed-like, or hypomanic-like episodes are substance or treatment induced or are due to the direct physiological effects of a general medical condition.

Recurrent

A. Presence of two or more Major Depressive Episodes.

Note: To be considered separate episodes, there must be an interval of at least 2 consecutive months in which criteria are not met for a Major Depressive Episode.

B. The Major Depressive Episodes are not better accounted for by Schizoaffective Disorder and are not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Psychotic Disorder Not Otherwise Specified.

C. There has never been a Manic Episode, a Mixed Episode, or a Hypomanic Episode.

Note: This exclusion does not apply if all the manic-like, mixed-like, or hypomanic-like episodes are substance or treatment induced or are due to the direct physiological effects or a general medical condition.

A 2 Known Risk Factors of Depression

(Adapted from Cheong, J. [147])

Family History – Having an immediate family member with depression or other mental illnesses.

Early Childhood Experiences -- Early childhood trauma, neglect, physical, emotional or sexual abuse, including parental divorce or loss of a parent.

Stress -- Negative life events such as, bereavement, divorce, loss of employment. Research has also shown that chronic stress may lead to depression.

Alcohol -- Depression and alcoholism are often found comorbidly.

Residence – Rates have been found to be higher in urban residents than in rural residents.

Marital Status -- Depression is highest among those being divorced, separated, or co-habiting. It is lowest among single and married persons. However, rates are higher in people living alone vs. those living with others.

Work Status – Unemployed persons (6 month duration) have been shown to have higher rates of depression than those employed.

Physical Illness -- Physical illnesses such as thyroid disorder, hormonal problems, chronic viral infections, cancer and heart diseases have been linked with depression.

Medications -- Some medications can cause depression-like symptoms, including sedatives, pain medications, some antihypertensives and long term corticosteroids.

Gender – It has been identified that a 2:1 ratio of women to men experience some kind of depression during their lifetimes. Women may suffer from unique forms of depression related to their unique biology and life experiences.

Age -- The average age of onset for depression is the mid-20s with most first episodes occurring between the age of 20 and 40. Research has shown that age of onset is decreasing.

Ethnic and Cultural Groups – All ethnic and cultural groups experience depression but cultural differences can affect the impact and expression of the disorder

Tobacco – Increased tobacco use is common in depressed patients

Appendix B- Assessment Tools

Childhood Trauma Questionnaire

Name: _____ **Age:** _____ **Sex:** _____

When I Was Growing up....	Never True	Rarely True	Some-times True	Often True	Very Often True
1. I didn't have enough to eat.	•	•	•	•	•
2. I knew that there was someone to take care of me and protect me.	•	•	•	•	•
3. People in my family called me things like "stupid," "lazy" or "ugly."	•	•	•	•	•
4. My parents were too drunk or high to take care of the family	•	•	•	•	•
5. There was someone in my family that helped me feel important or special.	•	•	•	•	•
6. I had dirty clothes to wear.	•	•	•	•	•
7. I felt loved.	•	•	•	•	•
8. I thought that my parents wished that I had never been born.	•	•	•	•	•
9. I got hit so hard by someone in my family that I had to go see a doctor or go to the hospital.	•	•	•	•	•
10. There was nothing that I wanted to change about my family.	•	•	•	•	•
11. People in my family hit me so hard that it left me with bruises or marks.	•	•	•	•	•
12. I was punished with a belt, a board, a cord, or some other hard object.	•	•	•	•	•
13. People in my family looked out for each other.	•	•	•	•	•
14. People in my family said hurtful or insulting things to me.	•	•	•	•	•
15. I believe that I was physically abused.	•	•	•	•	•
16. I had the perfect childhood.	•	•	•	•	•
17. I got hit or beaten so badly that it got noticed by someone like a teacher, neighbor, or doctor.	•	•	•	•	•
18. I felt that someone in my family hated me.	•	•	•	•	•
19. People in my family felt close to each other.	•	•	•	•	•
20. Someone tried to touch me in a sexual way, or tried to make me touch them.	•	•	•	•	•
21. Someone threatened to hurt me or tell lies about me, unless I did something sexual with them.	•	•	•	•	•
22. I had the best family in the world.	•	•	•	•	•
23. Someone tried to make me do sexual things or watch sexual things.	•	•	•	•	•
24. Someone molested me.	•	•	•	•	•
25. I believe that I was emotionally abused.	•	•	•	•	•
26. There was someone to take me to the doctor, if I needed it.	•	•	•	•	•
27. I believe that I was sexually abused.	•	•	•	•	•
28. My family was a source of strength and support.	•	•	•	•	•

B.0: The Childhood Trauma Questionnaire [118]

Appendix C – Preceding Studies

C 1 DTI Study

A DTI investigation was conducted prior to the anatomical MRI analysis carried out in the current study. The DTI investigation examined microstructural WM changes in MDD and their relationship to CIT. DTI tractography was used to examine preselected WM tracts (medial prefrontal [see Figure C.0] and medial occipital [not shown here]), with the hypothesis that changes would be more marked in patients with a history of CIT.

Acquisition

Twice refocused spin-echo echoplanar DTI at 1.5T was used to acquire axial-oblique images parallel to the AC-PC line. Parameters were designated as follows - TR = 10s; TE = 88 ms; 6 directions (1 0 1), (-1 0 1), (0 1 1), (0 1 -1), (1 1 0), (-1 1 0); b = 1,000 s/mm^2, FOV = 256 x 256mm; matrix = 128x128, zero-filled to 1.0x1.0 mm^2); 63 x 2.0 mm slices, no gap; 8 averages.

DTI analyses were conducted using DTI Studio software (John Hopkins, Baltimore). All voxels with FA $\geq$ 0.3 were seeded and propagated voxel-by-voxel until they reached a voxel with FA $<$ 0.3 or exceeded an angular deviation $>$ 70 degrees. ROIs were then placed around WM regions of interest to define the specific tracts. Medial prefrontal (forceps minor) and medial parieto-occipital WM were defined using ROIs surrounding the midline genu and splenium, respectively, of the CC, bounded by the coronal planes where their profiles meet the callosal body (see Figure C.0). A ventromedial portion of forceps minor was defined using an ROI one slice below the lower border of the genu and rostrum. The tract had to pass through this ROI as well as the CC. For the DTI study, FA was averaged from all voxels within the tracts. For the correlational analysis in the present thesis, FA was analyzed only from those voxels posterior to the anterior margin of the genu, excluding more peripheral WM.

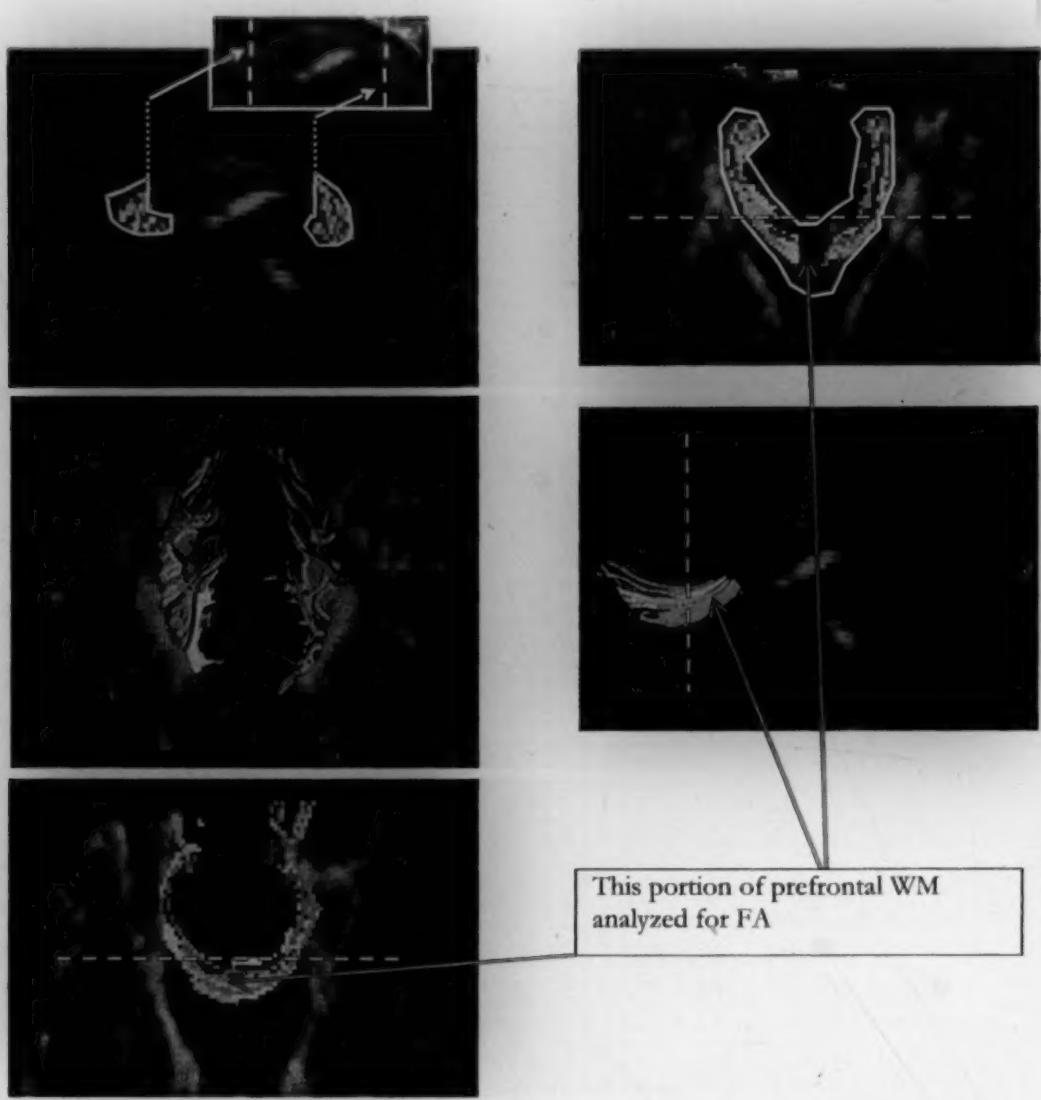

Figure C.0: Medial Prefrontal (left column) and Ventromedial (right column) White Matter

Results

Age, sex and education were not significant covariates of medial prefrontal or medial occipital FA and the data were therefore analyzed without covariates. Results showed a reduction of FA in these regions in MDD subjects (see Table C.0).

White matter tract	Fractional Anisotropy (FA)		df	F	p
	Control n = 28	MDD n = 29			
Prefrontal	.579 ± .016	.569 ± .018	1,55	5.16	.03
Ventromedial	.584 ± .019	.573 ± .016	1,55	4.76	.03
Occipital	.598 ± .013	.591 ± .015	1,55	4.02	.05

Table C.0: Comparison of Medial Prefrontal, Ventromedial and Occipital FA in Controls and MDD Subjects.

Subsequent analysis of MDD subgroups showed that the reduction in medial prefrontal FA was limited to MDD subjects with a history of childhood sexual/physical abuse (see Table C.1). A trend association was also found between medial prefrontal FA and childhood emotional abuse/neglect (see Table C.2).

White matter tract	Fractional Anisotropy (FA)			df	F	p
	Control n = 28	MDD, no SPA n = 13	MDD with SPA n = 16			
Prefrontal	.579 ± .016	.578 ± .018	.562 ± .014*	2,54	6.75	.002
Ventromedial	.584 ± .019	.577 ± .016	.570 ± .018†	2,54	2.81	.07
Occipital	.598 ± .013	.590 ± .014	.591 ± .017	2,54	1.97	.15

Table C.1: Comparison of Medial Prefrontal, Ventromedial and Occipital Fractional Anisotropy between Controls and MDD Subjects, with or without History of Childhood Sexual/Physical Abuse (SPA).

*MDD with SPA < controls, p = .001; MDD with SPA > MDD, no SPA, p = .008.
†MDD with SPA < controls, p = .02.

White matter tract	Fractional Anisotropy (FA)			df	F	p
	Control n = 28	MDD, no EAN n = 12	MDD with EAN n = 17			
Prefrontal	.579 ± .016	.568 ± .016*	.570 ± .019*	2,54	2.58	.09
Ventromedial	.584 ± .019	.571 ± .010†	.575 ± .020	2,54	2.53	.09
Occipital	.598 ± .013	.590 ± .015	.591 ± .016	2,54	1.97	.15

Table C.2: Comparison of Medial Prefrontal, Ventromedial and Occipital Fractional Anisotropy between Controls and MDD Subjects, with or without History of Emotional Abuse/Neglect (EAN).

*MDD with EAN < controls, p = .08; MDD, no EAN < controls, p = .06.
†MDD no EAN < controls, p = .045.

Reductions in medial prefrontal FA were found in both unmedicated and medicated MDD subjects (see Table C.3), but reduced occipital FA was limited to medicated subjects.

White matter tract	Fractional Anisotropy (FA)			df	F	P
	Control n = 34	MDD, unmedicated n = 14	MDD, medicated n = 15			
Prefrontal	.579 ± .016	.568 ± .014*	.570 ± .022	2,54	2.62	.08
Ventromedial	.584 ± .019	.572 ± .011	.574 ± .022	2,54	2.36	.10
Occipital	.598 ± .013	.595 ± .016	.586 ± .013†	2,54	3.50	.04

Table C.3: Comparison of Medial Prefrontal, Ventromedial and Occipital Fractional Anisotropy between Controls and Unmedicated or Medicated MDD Subjects.

*Unmedicated < controls, p = .04.
†Medicated MDD < controls, p = .01.

The FA results obtained in MDD subjects were further analyzed using ANOVA to test the main effects and interactions of childhood SPA and EAN. For medial prefrontal FA, a main effect of SPA was shown ($F_{1,25} = 7.31$; $p = 0.01$), but no main effect of EAN ($F_{1,25} = 0.97$; $p = 0.33$) or SPA x EAN interaction were present ($F_{1,25} = 1.09$; $p = 0.31$). For medial occipital FA, neither the main effects of SPA ($F_{1,25} = 0.02$; $p = 0.88$), EAN ($F_{1,25} = 0.01$; $p = 0.99$) or their interaction ($F_{1,25} = 0.04$; $p = 0.85$) were significant (see Table C.4).

Factors		n	Medial prefrontal FA	Medial occipital FA
No SPA	No EAN	7	.572 ± .019	.590 ± .017
	With EAN	6	.585 ± .015	.589 ± .008
With SPA	No EAN	6	.562 ± .009	.590 ± .013
	With EAN	11	.562 ± .017	.591 ± .017

Table C.4: Analysis of FA in MDD According to Subtypes of Childhood Maltreatment.

In summary, results showed a reduction of the FA of both tracts in MDD subjects, including the ventral portion of medial prefrontal FA, particularly in subjects with SPA. The trend reduction in medial prefrontal FA in patients with a history of EAN was due to their also having experienced SPA. Reductions in medial occipital FA were not associated with measures of childhood maltreatment.

C 2 Prefrontal Volume Study

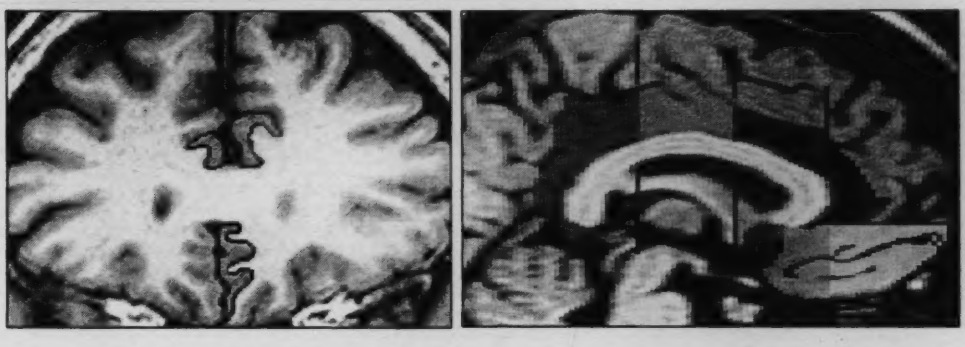

Figure C.1: Prefrontal Cortex Gray Matter

An accompanying investigation into ventromedial prefrontal cortex (vmPFC) GM volumes was carried out in the same subjects. ICV was a significant covariate of vmPFC volume ($F_{1,66}$ = 13.63; $p < .001$), but not age ($F_{1,66}$ = 1.81; $p = .18$), sex ($F_{1,66}$ = 0.42; $p = .52$) or education ($F_{1,66}$ = 2.34; $p = .13$). ICV was therefore included as a covariate in the model for subsequent analyses.

Group	n	vmPFC volume (mm^3)	df	F	p
Control	34	8,605 ± 1,389	1,69	0.07	.79
MDD	38	8,513 ± 1,850			
Control	34	8,603 ± 1,389	2,68	3.26	.045
MDD, no sexual/physical abuse	20	9,076 ± 1,849			
MDD with sexual/physical abuse	18	7,890 ± 1,626*			
Control	34	8,605 ± 1,389	2,68	1.55	.22
MDD, no emotional abuse/neglect	17	8,974 ± 1,856			
MDD with emotional abuse/neglect	21	8,136 ± 1,833			
Control	34	8,605 ± 1,389	2,68	0.21	.81
MDD, unmedicated	22	8,388 ± 1,754			
MDD, medicated	16	8,605 ± 1,389			

Table C.5: Comparison of vmPFC Volume between Controls and MDD Subjects or MDD Subgroups.

*MDD with SPA < MDD, no SPA, $p = .01$; MDD with SPA < control, $p = .09$.

The above analyses suggested a decrease in vmPFC volume associated with childhood sexual/physical abuse in MDD. Further analysis of MDD subjects was performed using ANCOVA, with factors of SPA and EAN, with ICV included as a covariate. This showed a trend effect of SPA ($F_{1,33} = 3.25$; $p = .08$), but no effect of EAN ($F_{1,33} = 1.28$; $p = .27$) or SPA x EAN interaction ($F_{1,33} = 0.61$; $p = .44$), although subjects with both types of abuse tended to have the smallest vmPFC volumes. Comparing MDD subjects with SPA alone and MDD subjects with both types of abuse to controls, subjects with SPA alone did not differ ($p = .99$), whereas subjects with both types of abuse had lower vmPFC volumes ($p = .02$).

Factors		n	vmPFC volume (mm^3)
No SPA	No EAN	11	9,208 ± 1,839
	With EAN	9	9,013 ± 1,971
With SPA	No EAN	6	8,637 ± 1,992
	With EAN	12	7,571 ± 1,379

Table C.6: Analysis of vmPFC Volume in MDD According to Subtypes of Childhood Maltreatment.

In summary, the analysis identified ICV as a significant correlate and suggested a decrease in vmPFC volume particularly associated with combined childhood SPA and EAN in MDD.

Appendix D- Other Figures and Tables

Figure D.0: Diffusion Tensor Imaging: Colour Maps of Callosal Organization (From Klein et al., [146])

Measure	Number Of Studies	Effect Size (Sex Difference M To F, Standard Deviation Units)	95% CI	
			Lower	Upper
Brain Weight	8	1.20	0.95	1.46
CC Area	41	0.21	0.13	0.29
Splenium Area	23	0.04	-0.08	0.16
Splenium/ CC Ratio	17	-0.11	-0.25	0.02
Splenium Width	23	-0.04	-0.04	-0.15
Isthmus Area	7	0.12	-0.06	0.30

Table D.0: Results of Meta-Analyses of Sex Differences in Corpus Callosum Literature (Summary of Bishop and Wahlsten, [39])